

FORM PTO-1390 (Modified) (REV 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 284.00010101	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR Unassigned 10/069605)	
INTERNATIONAL APPLICATION NO. PCT/US00/23482		INTERNATIONAL FILING DATE 26.08.00		PRIORITY DATE CLAIMED 26.08.99	
TITLE OF INVENTION PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS					
APPLICANT(S) FOR DO/EO/US Keith M. Skubitz et al.					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 11. <input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409). 12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210). 					
Items 13 to 20 below concern document(s) or information included:					
<ol style="list-style-type: none"> 13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 15. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 16. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 17. <input type="checkbox"/> A substitute specification. 18. <input type="checkbox"/> A change of power of attorney and/or address letter. 19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail 23. <input checked="" type="checkbox"/> Other items or information: 					
Application Data Sheet (4 pgs.); check in the amount of \$1,168.00 for filing fee; and itemized return-stamped postcard.					

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.492) 107069605		INTERNATIONAL APPLICATION NO. PCT/US00/23482		ATTORNEY'S DOCKET NUMBER 284.00010101	
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24. The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$710.00	
				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	45 - 20 =	25	x \$18.00	\$450.00	
Independent claims	17 - 3 =	14	x \$84.00	\$1,176.00	
Multiple Dependent Claims (check if applicable). <input type="checkbox"/>				\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$2,336.00	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$1,168.00	
SUBTOTAL =				\$1,168.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$1,168.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>				\$0.00	
TOTAL FEES ENCLOSED =				\$1,168.00	
				Amount to be refunded	\$
				charged	\$

a. ☒ A check in the amount of **\$1,168.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **13-4895**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Ann M. Mueting
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P.O. Box 581415
Minneapolis, Minnesota 55458-1415
United States of America

Ann M Mueting
 SIGNATURE

Ann M. Mueting
 NAME

33,977
 REGISTRATION NUMBER

Feb. 26, 2002
 DATE

Express Mail Label No.: EL659648471US
Date of Deposit: August 2, 2002

62A/ 10069605 .022602
Attorney Docket No. 25210-011 NATL
71
3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Skubitz, et al.
SERIAL NUMBER: 10/069,605 EXAMINER: Not yet assigned
FILING DATE: February 26, 2002 CONF NO.: 3442
FOR: PEPTIDES CAPABLE OF MODULATING THE FUNCTIONS OF CD66
(CEACAM) FAMILY MEMBERS

August 2, 2002
Boston, Massachusetts

BOX PCT
Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C. 20231

**PRELIMINARY AMENDMENT AND RESPONSE TO
NOTICE OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371
IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)**

Please amend the above-identified application as follows:

In the Specification:

Insert the enclosed Sequence Listing at the end of the specification.

REMARKS

In response to the June 4, 2002 Notification of Missing Requirements, Applicants submit herewith a Computer Readable Form (CRF) copy of the "Sequence Listing," a paper copy of the "Sequence Listing," and a statement that the content of the paper and computer readable copies are the same and include no new matter, in compliance with 37 C.F.R. §§ 1.821-1.825. The specification has been amended to insert the sequence listing. No new matter has been added.

Applicants: Skubitz, et al.
U.S.S.N. 10/023,601

The response is due on or before August 4, 2002. Applicants believe that no further fee is required for filing in the present submission. However, the Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Attorney Reference No. 25210-011 NATL. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at (212) 692-6785.

Respectfully submitted,


Ivor R. Elrifi, Reg. No. 39,529
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Dated: August 2, 2002

NYC 241959v1

191 PR75

10069610/069605
JC19 Rec'd PCT/PTO 26 FEB 2002

PATENT
Docket No. 284.0001 0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Keith N. Skubitz et al.)	Group Art Unit:	Unknown
)		
Serial No.:	Unassigned)	Examiner:	Unknown
)		
Filed:	Herewith)		
)		
For:	PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS			

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Attn: Box PCT
Washington, D.C. 20231

Dear Sir:

Prior to taking up the above-identified application for examination, please amend the application as follows:

In the Specification

Please replace the paragraph beginning at page 1, line 7, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Cross-Referenced to Related Applications

This application claims the benefit of United States Provisional Patent Application Serial Numbers 60/150,791 (filed 26 August 1999), 60/152,501 (filed 2 September 1999), and International Application No. PCT/US00/23482 (filed 26 August 2000) which are incorporated herein by reference.

Preliminary Amendment

Page 2

Applicant(s): Keith N. Skubitz et al.

Serial No.: Unassigned

Filed: Herewith

For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

In the Claims

Please amend claim 4. The amended claim is provided below in clean form. Per 37 C.F.R. §1.121, amended claim is also shown in Appendix A with notations to indicate changes made (for convenience, all pending claims, are provided in Appendix A).

4. (Amended) An isolated peptide from a surface exposed region of a CD66 family member

which is capable of modulating at least one of the following:

activation of neutrophils;

activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells;

proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells;

proliferation and/or differentiation of epithelial cells;

homotypic and/or heterotypic adhesion among CD66 family members; and

adhesion of CD66 family members to other ligands.

Preliminary Amendment

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Applicant(s): Keith N. Skubitz et al.

Serial No.: Unassigned

Filed: Herewith

For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

Remarks

The amendment made on page 1, line 7, were made in order to complete the priority information for the above-identified application.

The amendment made to claim 4 is supported in the specification at page 33, lines 30-31.

Conclusion

The Examiner is invited to contact Applicant's Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

Respectfully submitted for
Keith N. Skubitz et al.

By

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February 26, 2002

Date

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WASHINGTON, D.C. 20231

PRINTED NAME

Tim R Price

SIGNATURE

Tim R Price

**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE**

**Serial No.: Unassigned
Docket No.: 284.00010101**

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

The paragraph beginning at page 1, line 7, has been amended as follows:

Cross-Referenced to Related Applications

This application claims the benefit of United States Provisional Patent Application Serial Numbers 60/150,791 (filed 26 August 1999), 60/152,501 (filed 2 September 1999), and International Application No. PCT/US00/23482 (filed 26 August 2000) which are incorporated herein by reference.

In the Claims

For convenience, all pending claims are shown below.

1. An isolated peptide comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof that modulate the function of at least one CD66 family member and/or at least one ligand thereof.
2. The peptide of claim 1 represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, or 187.
3. The peptide of claim 2 represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54.
4. (Amended) An isolated peptide from a surface exposed region of a CD66 family member [The peptide of claim 1] which is capable of modulating at least one of the following:
 - activation of neutrophils;
 - activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells;

Preliminary Amendment - APPENDIX A

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Applicant(s): Keith N. Skubitz et al.

Serial No.: Unassigned

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proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells;

proliferation and/or differentiation of epithelial cells;

homotypic and/or heterotypic adhesion among CD66 family members; and adhesion of CD66 family members to other ligands.

5. The peptide of claim 1 which is complexed with a carrier molecule or structure to form a peptide conjugate.
6. The peptide of claim 5 wherein the carrier molecule or structure is selected from the group of microbeads, liposomes, biological carrier molecules, synthetic polymers, biomaterials, and cells.
7. The peptide of claim 6 wherein the peptide conjugate binds to cells expressing a CD66 protein or a CD66 ligand.
8. The peptide of claim 5 wherein the peptide conjugate includes a label.
9. The peptide of claim 1 which is attached to a label.
10. The peptide of claim 9 wherein the label is selected from the group consisting of a fluorescent tag, a radioactive tag, a magnetic resonance tag, an enzymatic tag, and combinations thereof.
11. A method of activating a neutrophil comprising contacting the neutrophil with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1, 2, 3, 4, 17, 41, or analogs thereof.

Preliminary Amendment - APPENDIX A

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Applicant(s): Keith N. Skubitz et al.

Serial No.: Unassigned

Filed: Herewith

For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

12. The method of claim 11 wherein the peptide is represented by SEQ ID NOs:1, 2, 3, 4, 17, or 41.
13. The method of claim 11 which is carried out *in vitro*.
14. The method of claim 11 which is carried out *in vivo*.
15. A method of blocking the activation of a neutrophil induced by the method of claim 11, the method comprising contacting the neutrophil when in the presence of at least one of the peptides listed in claim 11 with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, 84, or analogs thereof.
16. The method of claim 15 wherein the peptide is represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, or 84.
17. The method of claim 15 which is carried out *in vitro*.
18. The method of claim 15 which is carried out *in vivo*.
19. A method of modulating the homotypic and/or heterotypic adhesion of CD66 family members or adhesion of a CD66 protein to a CD66 ligand; the method comprising contacting CD66 family members and/or their ligands with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, 54, or analogs thereof.
20. The method of claim 19 wherein the peptide is represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54.

Preliminary Amendment - APPENDIX A

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Applicant(s): Keith N. Skubitz et al.

Serial No.: Unassigned

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For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

21. The method of claim 19 which is carried out *in vitro*.
22. The method of claim 19 which is carried out *in vivo*.
23. A method of altering the modulation of the homotypic and/or heterotypic adhesion of CD66 family members or adhesion between a CD66 protein and a CD66 ligand induced by the method of claim 19, the method comprising contacting CD66 family members and/or their ligands when in the presence of at least one of the peptides listed in claim 19 with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, 72-100, or analogs thereof.
24. The method of claim 23 wherein the peptide is represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, or 72-100.
25. The method of claim 23 which is carried out *in vitro*.
26. The method of claim 23 which is carried out *in vivo*.
27. A method of modulating immune cell activation, proliferation, and/or differentiation; the method comprising contacting an immune cell with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:14, 53, or analogs thereof.
28. The method of claim 27 wherein the peptide is represented by SEQ ID NOs:14 or 53.
29. The method of claim 27 wherein the immune cell is selected from the group of a T-cell, a B-cell, a LAK cell, an NK cell, a dendritic cell, and combinations thereof.

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Applicant(s): Keith N. Skubitz et al.

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For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

30. The method of claim 27 which is carried out *in vitro*.
31. The method of claim 27 which is carried out *in vivo*.
32. A method of modulating at least one of the following functions of CD66 family members and/or ligands thereof in cells: activation of neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of T-cells, B-cells, LAK cells, NK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of epithelial cells; homotypic and/or heterotypic adhesion among CD66 family members; and adhesion of CD66 family members to other ligands; the method comprising contacting cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
33. A method of delivering a therapeutically active agent to a patient comprising administering at least one peptide conjugate comprising a peptide and the therapeutically active agent to a patient wherein the peptide comprises an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
34. The method of claim 33 wherein the therapeutically active agent is selected from drugs, DNA sequences, RNA sequences, proteins, lipids, and combinations thereof.
35. The method of claim 33 wherein the therapeutically active agent is an antibacterial agent, antiinflammatory agent, or antineoplastic agent.
36. A method of modifying the metastasis of malignant cells comprising contacting the malignant cells or normal host tissue with at least one peptide or peptide conjugate

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Applicant(s): Keith N. Skubitz et al.

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For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

- comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
37. A method of altering bacterial or viral binding to cells or a biomaterial, the method comprising contacting the cells or biomaterial with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
38. A method of altering cell adhesion to a biomaterial, the method comprising contacting the biomaterial with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
39. A method of detecting tumors comprising contacting tumor cells or tumor vasculature with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
40. A method of detecting inflammation comprising contacting inflamed vasculature or leukocytes with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
41. A method of detecting a CD66 protein or a ligand thereof, the method comprising contacting tissue comprising a CD66 protein or a ligand thereof with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
42. A method of altering angiogenesis comprising contacting endothelial cells, tumor cells, or immune cells with at least one peptide or peptide conjugate comprising an

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Applicant(s): Keith N. Skubitz et al.

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For: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

43. A method of altering an immune response, the method comprising contacting immune system cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
44. A method of altering keratinocyte proliferation comprising contacting keratinocytes with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
45. An isolated peptide comprising an amino acid sequence represented by SMPFN (SEQ ID NO:101), PQQLF (SEQ ID NO:102), LPQQL (SEQ ID NO:103), QQLFG (SEQ ID NO:104), NRQIV (SEQ ID NO:105), GNRQI (SEQ ID NO:106), IKSDLVNE (SEQ ID NO:107), AASNPP (SEQ ID NO:108), NTTYLWWVNG (SEQ ID NO:109), YLWWVNG (SEQ ID NO:110), SWLIN (SEQ ID NO:111), SWFIN (SEQ ID NO:112), AQYSWLIN (SEQ ID NO:113), AQYSWFN (SEQ ID NO:114), SWFVN (SEQ ID NO:115), AQYSWFVN (SEQ ID NO:116), NRQII (SEQ ID NO:199), GNRQI (SEQ ID NO:200), or analogs thereof.

PTO/PCT Rec'd 23 FEB 2002

**PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66
(CEACAM) FAMILY MEMBERS**

Cross-Referenced to Related Applications

This application claims the benefit of United States Provisional Patent Application Serial Numbers 60/150,791 (filed 26 August 1999) and 60/152,501 (filed 2 September 1999), which are incorporated herein by reference.

Background of the Invention

CD66 family members appear to play a role in a wide variety of normal and pathological processes, including: cancer, embryonic development, bacterial infection, viral infection, inflammation, pregnancy, bile transport, and cell adhesion (1-3). CD66 monoclonal antibodies (mAbs) react with members of the carcinoembryonic antigen (CEA) family (4-13). In the CD terminology, mAbs belonging to the CD66 cluster are classified according to their reactivity with each family member, as indicated by a lower case letter after "CD66" as follows: CD66a, CEACAM-1 or biliary glycoprotein (BGP); CD66b, CEACAM-8 or CGM6; CD66c, CEACAM-6 or NCA; CD66d, CEACAM-3 or CGM1; CD66e, CEA; and CD66f, pregnancy specific glycoprotein (PSG) (13, 14). The CD66 (CEA) gene family belongs to the immunoglobulin (Ig) gene superfamily [for review see (1, 2, 15)]. Structurally, each of the human CD66 family members contains one amino-terminal (N) domain of 108-110 amino acid residues, homologous to Ig variable domains, followed by a differing number (0-6) of Ig C2-type constant-like domains. The structure of the N-domain is therefore predicted to be a stacked pair of beta-sheets with 9 beta-strands (16).

CD66 family members may potentially function as adhesion molecules (12, 17-30). CD66a, CD66c, and CD66e are capable of homotypic and heterotypic adhesion, as shown by use of recombinant CD66a which undergoes homotypic adhesion as well as heterotypic adhesion with CD66c and CD66e (1, 2, 4-12, 17-32). Data also suggest that CD66a plays a signaling role and

regulates the adhesion activity of CD11/CD18 in human neutrophils (8, 11, 25-27, 33, 34). CD66a, CD66b, CD66c, and CD66d, but not CD66e, are expressed in human neutrophils, where they are "activation antigens" in that their surface expression is increased following neutrophil stimulation with various stimuli.

- 5 CD66a, CD66b, CD66c, and CD66d mAb binding to the neutrophil surface triggers a transient activation signal that regulates the adhesive activity of CD11/CD18, and increases neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) (8, 11, 25-27, 33, 34).

- CD66a is frequently down regulated in colon, prostate, breast, and
10 hepatocellular carcinoma, and colorectal adenomas (35-39). Transfection studies have provided evidence that CD66a may act as a tumor suppressor in models of colon cancer (40) prostate cancer (41, 42) breast cancer (43) and bladder cancer (44). CD66a is also important in bacterial infections, since over 95% of pathogenic *N. meningitidis* and *N. gonorrhea* interact with CD66a via
15 Opa proteins, and the binding site for these Opa proteins has been localized to the N-domain of CD66a (45-50). An important property of Opa proteins is the stimulation of adhesion and non opsonic phagocytosis of Opa+ bacteria by neutrophils (reviewed in 48). CD66a also appears to function as a receptor for murine hepatitis virus (51-55). Furthermore, CD66a may play a role in
20 angiogenesis since it is selectively expressed on certain endothelial cells (56) and CD66a appears to function as a regulator of bile transport in bile canaliculi (3, 57, 58).

- The mechanism(s) by which CD66a transmits signals (e.g. activation in neutrophils, or growth regulating signals in epithelial cells and carcinomas) are
25 unclear. However, CD66a is phosphorylated on its cytoplasmic domain, largely on tyrosine with a lower level of phosphoserine, in neutrophils and colon cancer cells (4, 59-61). While at least eight isoforms of CD66a derived from differential splicing have been described (3, 12, 13, 25), only those isoforms with a long cytoplasmic tail can be phosphorylated on tyrosine. In addition,
30 associated protein tyrosine kinase and phosphatase activities may be involved in CD66a signaling (59, 62, 63).

Summary of the Invention

The present invention relates to peptides capable of modulating the function (e.g., signaling or adhesive activities) of CD66 (CEACAM) family members and/or their ligands. The sequences of these peptides are set forth in Tables I-IX. Active peptides (i.e., those capable of modulating the function of at least one CD66 family member and/or at least one ligand thereof) could be larger or smaller than the ones described here. While the present peptides described are of about 14 amino acids, peptides containing a relatively large number of amino acid residues, e.g., up to about 100 amino acid residues or more, that contain the described peptides, portions thereof, or similar peptides may have biological activity as well. Similarly, peptides smaller than those shown in Tables I-IX may also have similar biological activity. Similarly, peptides with amino acid substitutions or other alterations may block the activities of the described peptides or the parent molecules. Cyclic or otherwise modified forms of the peptides would also be expected to have biological activity.

Thus, the present invention provides isolated peptides that include an amino acid sequence represented by SEQ ID NOs:1-100 or analogs thereof that modulate the function of at least one CD66 protein (i.e., CD66 family member) and/or at least one ligand thereof. These amino acid sequences can form a part of a larger peptide. Additionally, they can be used in various combinations in any one peptide. Preferably, the present invention provides isolated peptides represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54. It is believed that SEQ ID Nos:119, 143, 157, 161, 178, and 187 would have activity if they were solubilized or conjugated in a complex.

A preferred group of isolated peptides include those having an amino acid sequence represented by SMPFN (SEQ ID NO:101), PQQLF (SEQ ID NO:102), LPQQL (SEQ ID NO:103), QQLFG (SEQ ID NO:104), NRQIV (SEQ ID NO:105), GNRQI (SEQ ID NO:106), IKSDLVNE (SEQ ID NO:107), AASNPP (SEQ ID NO:108), NTTYLWWVNG (SEQ ID NO:109), YLWWVNG (SEQ ID NO:110), SWLIN (SEQ ID NO:111), SWFIN (SEQ ID NO:112), AQYSWLIN (SEQ ID NO:113), AQYSWFIN (SEQ ID NO:114),

SWFVN (SEQ ID NO:115), AQYSWFVN (SEQ ID NO:116), NRQII (SEQ ID NO:199), GNRQI (SEQ ID NO:200), or analogs thereof. It is believed that these portions of certain of the peptides described herein contribute significantly to the activity of the peptides.

5 The present peptides are preferably capable of altering signaling mediated in part by CD66 (CEACAM) family members. Preferably, the peptides of the present invention modulate at least one of the following (which are functions of CD66 proteins and/or ligands thereof): activation of neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK cells,
10 dendritic cells, or other immune system cells; proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of epithelial cells such as breast or intestinal/colonic epithelium cells or keratinocytes. In addition these peptides are preferably capable of altering homotypic and/or heterotypic adhesion among
15 CD66 proteins (i.e., CD66 family members) or adhesion of CD66 proteins to other CD66 ligands.

 The present invention also provides peptide conjugates. The ability of peptides complexed with carrier molecules or structures, such as microbeads, liposomes, biological carrier molecules, synthetic polymers, biomaterials, and
20 cells, thereby forming peptide conjugates is shown to impart the larger structure with the ability to bind to cells expressing the appropriate CD66 family member. Such peptide conjugates bind to cells expressing a CD66 protein or a CD66 ligand.

 The peptides or peptide conjugates of the present invention can also
25 include molecules for labeling (i.e., labels such as fluorescence tags, magnetic resonance tags, radioactive tags, enzymatic tags). In this way, these can be used in diagnostic methods to detect specific targets *in vivo* or *in vitro*.

 The present invention provides a method of activating a neutrophil that includes contacting the neutrophil with a peptide or peptide conjugate (i.e., at
30 least one peptide or peptide conjugate) that includes an amino acid sequence represented by SEQ ID NOs:1, 2, 3, 4, 17, 41, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:1, 2, 3, 4, 17, or 41.

The present invention also provides a method of modulating the homotypic and/or heterotypic adhesion of CD66 family members or adhesion of a CD66 protein to a CD66 ligand. The method includes contacting CD66 family members and/or their ligands with a peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, 54, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54.

The present invention also provides a method of modulating (e.g., activating or inhibiting) immune cell (e.g., T-cells, B-cells, NK cells, LAK cells, or dendritic cells) activation, proliferation, and/or differentiation that includes contacting an immune cell with a peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:14, 53, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:14 or 53.

In addition, some peptides differ from these peptides by one or several amino acids and could compete with these active peptides or the natural CD66 family member or ligand thereof for certain biological activities.

For example, the present invention provides a method of blocking the activation of a neutrophil induced by the method described above. This method includes contacting the neutrophil when in the presence of at least one of the peptides used in the method of activating a neutrophil discussed above with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs: 18-21, 28-31, 39, 40, 55-59, 68-71, 84, or analogs thereof. Preferably, the peptide is represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, or 84.

As another example, the present invention provides a method of altering the modulation of the homotypic and/or heterotypic adhesion of CD66 family members or adhesion between a CD66 protein and a CD66 ligand induced by peptides homologous to (e.g., peptides derived from similar regions of similar domains of CD66 family members) those listed above (SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54). The method includes contacting CD66 family members and/or ligands thereof with a peptide comprising an amino acid sequence represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, 72-100, or analogs thereof, when in

the presence of at least one of the peptides listed above (SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54). Preferably, the peptide is represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, or 72-100. This modulating effect can result, for example
5 from direct binding of one of these peptides to one of the CD66 family members and/or ligands thereof, or from altering the effects of other peptides on the adhesion.

Another method of the present invention involves modulating at least one of the following functions of CD66 family members and/or ligands thereof
10 in cells: activation of neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of T-cells, B-cells, LAK cells, NK cells, dendritic cells, or other immune system cells; proliferation and/or differentiation of epithelial cells; homotypic and/or heterotypic adhesion among CD66 family members;
15 and adhesion of CD66 family members to other ligands. The method includes contacting cells with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves delivering a therapeutically active agent to a
20 patient. The method includes administering at least one peptide conjugate comprising a peptide and the therapeutically active agent to a patient wherein the peptide includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof. Preferably, the therapeutically active agent is selected from drugs, DNA sequences, RNA
25 sequences, proteins, lipids, and combinations thereof. More preferably, the therapeutically active agent is an antibacterial agent, antiinflammatory agent, or antineoplastic agent.

There is also provided a method of modifying the metastasis of malignant cells. This method includes contacting the malignant cells or normal
30 host tissue with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

There is also provided a method of altering bacterial or viral binding to cells or a biomaterial. The method includes contacting the cells or biomaterial with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves altering cell adhesion to a biomaterial. The method includes contacting the biomaterial with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves detecting tumors. The method includes contacting tumor cells or tumor vasculature with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Still another method involves detecting inflammation. The method includes contacting inflamed vasculature or leukocytes with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Yet another method involves detecting a CD66 protein or a ligand thereof. The method includes contacting tissue containing a CD66 protein or a ligand thereof with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method involves altering angiogenesis. The method includes contacting endothelial cells, tumor cells, or immune cells with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Yet another method of the present invention involves altering an immune response. The method includes contacting immune system cells with at least one peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

Another method of the present invention involves altering keratinocyte proliferation. The method includes contacting keratinocytes with at least one

peptide or peptide conjugate that includes an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

The methods described herein can be carried out *in vitro* or *in vivo*. The peptides can be used alone or in various combinations as well as in peptide
5 conjugates. They are used in amounts that provide the desired effect. These amounts can be readily determined by one of skill in the art. Preferably, for each of the methods of the present invention, useful peptides are represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54.

As used herein, "a" or "an" refers to one or more of the term modified.

10 Thus, the compositions and methods of the present invention include one or more polypeptides. Also, herein when peptide is said to includes an amino acid sequence represented by SEQ ID NOs:1-100 or analogs thereof, the peptide can include one or more of the sequences specified.

"Amino acid" is used herein to refer to a chemical compound with the
15 general formula: $\text{NH}_2\text{-CRH-COOH}$, where R, the side chain, is H or an organic group. Where R is an organic group, R can vary and is either polar or nonpolar (i.e., hydrophobic). The amino acids of this invention can be naturally occurring or synthetic (often referred to as nonproteinogenic). As used herein, an organic group is a hydrocarbon group that is classified as an aliphatic group,
20 a cyclic group or combination of aliphatic and cyclic groups. The term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic
25 group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" refers to mono- or polycyclic aromatic hydrocarbon groups. As used herein, an organic group can be substituted or unsubstituted.

The terms "polypeptide" and "peptide" as used herein, are used
30 interchangeably and refer to a polymer of amino acids. These terms do not connote a specific length of a polymer of amino acids. Thus, for example, the terms oligopeptide, protein, and enzyme are included within the definition of polypeptide or peptide, whether produced using recombinant techniques,

chemical or enzymatic synthesis, or naturally occurring. This term also includes polypeptides that have been modified or derivatized, such as by glycosylation, acetylation, phosphorylation, and the like.

Herein, "isolated" as it refers to peptides (i.e., polypeptides) means that the peptides are derived from naturally occurring proteins or other polypeptides and free from other biological material or they are synthesized, either recombinantly or chemically.

The following abbreviations are used throughout the application:

A = Ala = Alanine	T = Thr = Threonine
V = Val = Valine	C = Cys = Cysteine
L = Leu = Leucine	Y = Tyr = Tyrosine
I = Ile = Isoleucine	N = Asn = Asparagine
P = Pro = Proline	Q = Gln = Glutamine
F = Phe = Phenylalanine	D = Asp = Aspartic Acid
W = Trp = Tryptophan	E = Glu = Glutamic Acid
M = Met = Methionine	K = Lys = Lysine
G = Gly = Glycine	R = Arg = Arginine
S = Ser = Serine	H = His = Histidine

Brief Description of the Drawings

Figure 1. Effects of CD66a peptides on neutrophil adhesion to human umbilical vein endothelial cells (HUVECs). HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by adding 50 ng/ml TNF-alpha and 1000 U/ml gamma-interferon (gamma-IFN) and culturing for 48 hours. The wells were then washed and 25 µl of adhesion buffer containing the indicated CD66a peptide at 167 µg/ml (final concentration) was added. One hundred µl of adhesion media containing 10^5 neutrophils labeled with calcein AM was then immediately added, followed by 25 µl of adhesion buffer without (solid bars) or with (hatched bars) 6×10^{-7} M formyl-met-leu-phe (FMLP), and the plates were incubated at 37°C for 30 min in 5% CO₂. The wells were then

washed and the number of adherent neutrophils determined with a fluorescence plate reader. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means \pm SD of 4 separate determinations. The adhesion observed in the presence of the active CD66a peptides CD66a-1, CD66a-2, and CD66a-3 was statistically greater than that observed with 24 other peptides or media alone ($p < 0.05$).

Figure 2. Effects of various concentrations of the CD66a peptides CD66a-1, CD66a-2, and CD66a-3 on neutrophil adhesion to HUVECs.

HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by incubating in the presence of TNF-alpha at 50 ng/ml final concentration for 4 hr at 37°C, and the adhesion of neutrophils was quantitated in the presence of the indicated final concentration of CD66a peptide CD66a-1 (circles), CD66a-2 (squares), or CD66a-3 (triangles) and 10^{-7} M FMLP as described in Figure 1. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means \pm SD of 4 separate determinations. The adhesion observed in the presence of CD66a peptides CD66a-1, CD66a-2, and CD66a-3 at 50 μ g/ml was statistically greater than that observed with lower concentrations of peptides ($p < 0.05$).

Figure 3. Effects of scrambled CD66a peptides on neutrophil adhesion to HUVECs. HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by incubating in the presence of TNF-alpha at 50 ng/ml final concentration for 4 hr at 37°C. The wells were then washed and 25 μ l of adhesion buffer containing the indicated CD66a peptides (at 167 μ g/ml final concentration) was added. One hundred microliters of adhesion media containing 10^5 neutrophils was then added, followed by 25 μ l of adhesion buffer without (solid bars) or with (hatched bars) 6×10^{-7} M FMLP, and the plates were incubated at 37°C for 30 min in 5% CO₂. The wells were then washed and the number of adherent neutrophils determined with a fluorescence plate reader as in Figure 1. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means \pm SD of 4 separate determinations. The adhesion observed in the presence of the active CD66a peptides CD66a-1, CD66a-2, and CD66a-3, were statistically greater than that observed with the 9 scrambled peptides ($p < 0.05$).

Figure 4. Representative flow cytometric histogram profiles of the effect of CD66a peptides on human neutrophil surface CD11b and CD62L expression. Left panel: Purified neutrophils were incubated with Hanks' balanced salt solution (HBSS) (mean channel fluorescence (MCF) = 584) (top panel), FMLP (10^{-7} M), (MCF = 709) (second panel), the CD66a peptide CD66a-1 (MCF = 704) (167 μ g/ml) (third panel), the CD66a peptide CD66a-2 (MCF = 713) (167 μ g/ml) (fourth panel), the CD66a peptide CD66a-3 (MCF = 714) (167 μ g/ml) (fifth panel), or the scrambled CD66a peptide CD66a-1-S1 (MCF = 581) (167 μ g/ml) (bottom panel) for 15 min at 37°C, and the binding of a phycoerythrin-labeled (PE-labeled) CD11b mAb was determined. Vertical axis, relative cell number; horizontal axis, relative fluorescence intensity measured on a log scale. The MCFs represent the means of two determinations that agreed within 10%. Right panel: Purified neutrophils were warmed to 37°C, incubated for 5 min with HBSS (MCF = 548) (top panel), FMLP (10^{-7} M), (MCF = 256) (second panel), the CD66a peptide CD66a-1 (MCF = 230) (167 μ g/ml) (third panel), the CD66a peptide CD66a-2 (MCF = 243) (167 μ g/ml) (fourth panel), the CD66a peptide CD66a-3 (MCF = 229) (167 μ g/ml) (fifth panel), or the scrambled CD66a peptide CD66a-1-S1 (MCF = 546) (167 μ g/ml) (bottom panel), and the binding of a PE-labeled CD62L mAb was determined. Vertical axis, relative cell number; horizontal axis, relative fluorescence intensity measured on a log scale. A duplicate experiment gave similar results.

Figure 5. Effects of CD66a-7 peptide on neutrophil adhesion to HUVECs. HUVECs were grown to confluence in 96 well microtiter plates, and stimulated by adding 50 ng/ml TNF-alpha and culturing for 48 hours. The wells were then washed and 25 μ l of adhesion buffer with or without the CD66a-7 peptide at 167 μ g/ml (final concentration) was added. One hundred microliters (μ l) of adhesion media containing 10^5 neutrophils was then immediately added, followed by 25 μ l of adhesion buffer with 6×10^{-7} M FMLP, and the plates were incubated at 37°C for 30 min in 5% CO₂. The wells were then washed and the number of adherent neutrophils determined with a fluorescence plate reader. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means \pm SD of 4 separate

determinations. The adhesion observed in the presence of the peptide CD66a-7 was statistically greater than that observed with buffer alone ($p < 0.05$).

Figure 6. Effects of CD66a-6L peptide on neutrophil adhesion to HUVECs. Using the method described with respect to Figure 5, the adhesion observed in the presence of the peptide CD66a-6L was statistically greater than that observed with buffer alone ($p < 0.05$).

Figure 7. Effects of CD66e-3 peptide on neutrophil adhesion to HUVECs. Using the method described with respect to Figure 5, the adhesion observed in the presence of the peptide CD66e-3 was statistically greater than that observed with buffer alone ($p < 0.05$).

Figure 8. Effects of CD66a peptides on binding of CHO transfectants expressing CD66a (CEACAM1-4L) to immobilized recombinant human CEACAM1-Fc using the technique of the Transfectant Binding Assay #1 (Assay #1). Ninety-six well Immulon 3 plates were coated with goat anti-human Fc, washed, and soluble CEACAM1-4-Fc (CD66a-Fc), or the negative control constructs CD31(D1-3)-Fc and CD14-Fc were added and allowed to bind, and the plates were then washed. CHO transfectants were labeled with the fluorescent tag BCECF-AM and allowed to adhere to these immobilized soluble constructs for 60 min at 37°C. The total fluorescence of each well was then determined using the Cytofluor II fluorescence plate reader. The plates were then washed and the number of cells adhering determined by fluorescence measurements in the Cytofluor II as a percentage of the total cells added per well. The mean \pm SD of 4 determinations are shown. Four of the peptides, CD66-17, CD66-18, CD66-19, and CD66-24, significantly inhibited homotypic CD66a binding in this assay.

Figure 9A and 9B. Effects of peptides on homotypic adhesion of CD66a-CD66a using Transfectant Binding Assay #2 (Assay #2). Several peptides blocked binding of CD66a expressing CHO transfectants to immobilized CD66a using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66a protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 10A and 10B. Effects of peptides on homotypic adhesion of CD66c-CD66c using Assay #2. Several peptides blocked binding of CD66c expressing CHO transfectants to immobilized CD66c using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66c protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 11A and 11B. Effects of peptides on homotypic adhesion of CD66e-CD66e using Transfectant Binding Assay #2 (Assay #2). Several peptides blocked binding of CD66e expressing CHO transfectants to immobilized CD66e using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66e protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 12A and 12B. Effects of peptides on heterotypic adhesion of CD66b-CD66c using Assay #2. Several peptides blocked binding of CD66b expressing CHO transfectants to immobilized CD66c using the technique of Assay #2. The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66c protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody.

Figure 13A and 13B. Binding of microbeads coupled to CD66a-24 to CHO cells expressing CD66a. CD66a-24 and CD66a-1 peptides were coupled to microbeads and the microbeads were incubated with a suspension of CHO cells expressing CD66a at room temperature for 30 min. The binding of the microbeads to the CHO cells was quantified by counting the number of beads associated with single cells or groups of cells in three cell-group size classes and are reported as the number of microbeads bound to each size group of cells. Figure 13B shows the number of beads associated with single cells, which are reported as the average number of microbeads bound to each single cell.

Figure 14. Effect of CD66 peptides on the activation of T-cells. T-cells were stimulated with anti-CD3 in the presence of various CD66 peptides as indicated and proliferation quantitated using radionucleide uptake expressed as cpm associated with the cells. Peptide CD66a-24, and to a lesser extent CD66e-31, inhibited T-cell activation.

Detailed Description of Preferred Embodiments of the Invention

Because of the adhesive and signaling properties of CD66a described above, we sought to identify functionally active domains of CD66a by use of synthetic peptides. Peptides of 14 amino acids in length were synthesized. The sequences are set forth in Tables I-IX. These were investigated for the ability to modulate the function of CD66 (CEACAM) family members. Thus, the present invention provides isolated peptides that include an amino acid sequence represented by (at least one of) SEQ ID NOs:1-100 or analogs thereof that modulate the function of at least one CD66 protein (i.e., CD66 family member) and/or at least one ligand thereof.

Peptides were tested for their ability to alter neutrophil adhesion to human umbilical vein endothelial cells (HUVECs). Five peptides activated neutrophils for adhesion to endothelial cells, as determined by increasing neutrophil adhesion to HUVEC monolayers and altering surface expression of CD11/CD18 and CD62L. The data suggest that at least 5 peptide motifs from the N-terminal domain of CD66a are involved in the interaction of CD66a with other ligands, and can initiate signal transduction in neutrophils. These 5 motifs have the amino acid sequences represented by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:17. Activating or inhibiting neutrophil activation may be useful in treating certain infectious diseases or in cases where the activation of neutrophils results in unwanted effects as in adult respiratory distress syndrome.

Similar modeling was done with CD66b, CD66c, CD66d, CD66e, and CD66f and peptides shown in Tables III-VIII were synthesized. One of these peptides was also found to activate neutrophils. This peptide has the sequence represented by SEQ ID NO:41. In addition, it was found that peptides from homologous regions of other CD66 members that contained minor amino acid

differences from the active peptides from CD66a did not activate neutrophils, thus providing further information on the structure needed for activity. These include peptides having the amino acid sequences represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, and 84. These peptides could compete
5 with the active peptides (SEQ ID NOs:1-4, 17, and 41) or could mediate direct binding of natural CD66 family members.

These peptides were also tested for their ability to alter the homotypic adhesion of CD66a to CD66a, CD66c to CD66c, and CD66e to CD66e, as well as the heterotypic adhesion of CD66b to CD66c. A number of the peptides
10 were found to modulate homotypic and/or heterotypic adhesion of CD66 family members. These include peptides having the amino acid sequences represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, and 54. It is believed that these may also modulate adhesion between a CD66 protein and other CD66 ligands. In addition, some peptides that differ from these active peptides by one
15 or several amino acids could compete (i.e., alter their modulation effects) with these active peptides for functional effects or mediate direct binding of the natural CD66 family members. These include peptides having the amino acid sequences represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, and 72-84, as well as other
20 homologous peptides (based on domain structure) including SEQ ID NOs:85-100.

Peptides were also tested for their ability to inhibit the activation of T-cells toward proliferation and/or differentiation. One peptide (SEQ ID NO:14) was found to be a potent inhibitor of T-cell activation while another (SEQ ID
25 NO:53) had weaker activity. Modulating the immune response, as for example by activating or inhibiting the proliferation and/or differentiation of T-cells, B-cells, NK cells, LAK cells, dendritic cells, or other immune system cells, may be useful in treating autoimmune diseases, and in transplantation therapies where graft vs. host or host vs. graft effects may be undesirable. The peptides
30 could also be immune stimulants in settings such as cancer, infectious disease, or immunization. Alternatively, they could be immune suppressants. They could also be used to detect inflammation, and preferably modulate inflammation by activating or inhibiting activation of immune or inflammatory

cells. A preferred method involves detecting (and preferably modulating) inflammation in tissues such as inflamed vasculature or leukocytes.

Thus, preferably, the present invention provides isolated peptides represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17, 22, 32, 33, 35, 37, 39, 41, 47, 53, or 54. It is also believed that peptides represented by SEQ ID Nos:119, 143, 157, 161, 178, and 187 would have activity if they were solubilized or conjugated in a complex.

Thus, the present invention provides peptides derived from CD66 (CEACAM) family members that are capable of modulating (i.e., altering by increasing, decreasing, etc.), for example, cell activation, cell adhesion, cell proliferation, cell differentiation, or homotypic and/or heterotypic adhesion among CD66 family members or binding of CD66 family members to their ligands.

In addition to the peptides discussed above that are specifically shown to have such activity, others are believed to possess a least one activity as described herein. These peptides are shown in Tables I-IX.

Compositions comprising the polypeptides of this invention can be added to cells in culture (*in vitro*) or used to treat patients, such as mammals (*in vivo*). Where the polypeptides are used to treat a patient, the polypeptide is preferably combined in a pharmaceutical composition with a pharmaceutically acceptable carrier such as a larger molecule to promote polypeptide stability or a pharmaceutically acceptable buffer that serves as a carrier for the polypeptide or incorporated in a peptide conjugate that has more than one peptide coupled to a single entity.

Given the known bacterial and viral binding properties of CD66 family members, the peptides described herein could be useful for altering the binding of viruses, bacteria, or other pathological etiologic agents to the cells of host tissues, transplanted tissues, or to biomaterials (increase or inhibit binding). They could also be useful for detecting a CD66 protein or a ligand thereof in tissue, whether it be *in vitro* or *in vivo*.

Studies were also performed to demonstrate that these peptides could be used to target the binding of larger structures to cells expressing the appropriate CD66 family member. The coupling of multiple copies of peptides to larger

structures (thereby forming peptide conjugates) allows cooperativity of binding due to the presence of multiple binding sites. This markedly increases the affinity of binding of the complex compared with that of a single free peptide. In addition, it should therefore be possible to complex various combinations and
5 densities of different peptides described herein to create a structure that preferentially binds cells expressing a specific pattern of CD66 family members.

The biological activity of the peptides identified here suggests that they have sufficient affinity to make them potential candidates for drug localization
10 to cells expressing the appropriate surface structures. This targeting and binding to cells could be useful for the delivery of therapeutically active agents (including targeting drugs, DNA sequences, RNA sequences, lipids, proteins (e.g., human growth factors)) and gene therapy/gene delivery. More preferably, the therapeutically active agent is an antibacterial agent, antiinflammatory
15 agent, or antineoplastic agent.

Since different cells, including specifically many malignant cells, cells of different tissues, growing endothelial cells, including endothelial cells in new vessels in tumors and in diabetic proliferative microvasculature, express different combinations of CD66 family members, it should be possible to
20 generate compounds bearing different combinations of densities of CD66 peptides that would target (bind preferentially) to different desired tissues or cells.

As proof of principle, the peptide CD66-24 when coupled to microbeads directs the binding of the complexed microbeads to CHO cells expressing
25 CD66a.

Also, CD66 family members have been shown to alter metastases of malignant cells and can alter cell differentiation. Thus, the peptides described herein could modify the process of metastasis of malignant cells either by altering the behavior of the malignant cells directly, or by altering the
30 physiology of a target tissue (as for example, the liver where CD66e has been shown to alter cytokine production by cells in the liver and also alter the ability of colon cancer cells to metastasize to the liver). The peptides described herein can also be used in detecting tumors.

Thus, the peptides described herein are believed to be useful for altering angiogenesis. In such a method, endothelial cells, tumor cells, or immune cells are contacted with at least one peptide described herein.

Some CD66 members are expressed in growing keratinocytes at the edge of healing wounds. These peptides may be useful to alter keratinocyte growth or behavior or the behavior of other cell involved in wound healing.

These peptides may be useful in altering the growth or physiology of cells, which are in various disease states, that can express CD66 members, including gut (as for example in inflammatory bowel disease, atrophic states, or cancer), breast, stomach, small bowel, colon, pancreas, thyroid, prostate, lung, kidney, placenta, sebaceous glands, and uterus.

Treatment for these various conditions can be prophylactic or therapeutic. Thus, treatment can be initiated before, during, or after the development of the condition. As such, the phrases "inhibition of" or "effective to inhibit" a condition includes both prophylactic and therapeutic treatment (i.e., prevention and/or reversal of the condition).

Additionally, molecules/particles with a specific number of specific CD66 peptides would bind specifically to cells/tissues expressing specific ligand combinations, and therefore could have diagnostic and therapeutic use.

Thus, the peptides of the present invention can be labeled (e.g., fluorescent, radioactive, enzyme, nuclear magnetic) and used to detect specific targets *in vivo* or *in vitro* including "immunochemistry" like assays *in vitro*. *In vivo* they could be used in a manner similar to nuclear medicine imaging techniques to detect tissues, cells, or other material expressing specific CD66 ligands.

The polypeptides of SEQ ID NOs:1-100 can be in their free acid form or they can be amidated at the C-terminal carboxylate group. The present invention also includes analogs of the polypeptide of SEQ ID NOs:1-100, which typically have structural similarity with SEQ ID NOs:1-100. An "analog" of a polypeptide includes at least a portion of the polypeptide, wherein the portion contains deletions or additions of one or more contiguous or noncontiguous amino acids, or containing one or more amino acid substitutions. Substitutes for an amino acid in the polypeptides of the invention are preferably conservative substitutions, which are selected from other members of the class to which the

amino acid belongs. An analog can also be a larger peptide that incorporates the peptides described herein. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can generally be substituted for another amino acid without substantially altering the structure of a polypeptide.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro; Class II: Cys, Ser, Thr, and Tyr; Class III: Glu, Asp, Asn, and Gln (carboxyl group containing side chains); Class IV: His, Arg, and Lys (representing basic side chains); Class V: Ile, Val, Leu, Phe, and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr, and His (representing aromatic side chains). The classes also include other related amino acids such as halogenated tyrosines in Class VI.

Polypeptide analogs, as that term is used herein, also include modified polypeptides. Modifications of polypeptides of the invention include chemical and/or enzymatic derivatizations at one or more constituent amino acid, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and the like.

A preferred polypeptide analog is characterized by having at least one of the biological activities described herein. Such an analog is referred to herein as a "biologically active analog" or simply "active analog." The biological activity of a polypeptide can be determined, for example, as described in the Examples Section.

For example, active analogs of SEQ ID NO:1 include peptides having an "M" or similar amino acid in the "SMPFN" sequence (SEQ ID NO:101). Active analogs of SEQ ID NO:2 include peptides having a "Q" or similar amino acid in the "PQQLF" sequence (SEQ ID NO:102), the "LPQQL" sequence (SEQ ID NO:103), or the "QQLFG" sequence (SEQ ID NO:104). Active analogs of SEQ ID NO:3 include peptides having an "RQ" sequence or similar

amino acid sequence in the "NRQIV" sequence (SEQ ID NO:105) or the "GNRQI" sequence (SEQ ID NO:106). Active analogs of SEQ ID NO:4 include peptides having an "IKSDLVNE" portion (SEQ ID NO:107) of the sequence. Active analogs of SEQ ID NO:9 include peptides having an "AASNPP" portion (SEQ ID NO:108) of the sequence. Active analogs of SEQ ID NO:22 include peptides having a "NTTYLWWVNG" portion (SEQ ID NO:109) or "YLWWVNG" portion (SEQ ID NO:110) of the sequence. Active analogs of SEQ ID NO:35 include peptides having an "SWLIN" portion (SEQ ID NO:111), "SWFIN" portion (SEQ ID NO:112), "AQYSWLIN" portion (SEQ ID NO:113), or "AQYSWFIN" portion (SEQ ID NO:114) of the sequence. Active analogs of SEQ ID NO:47 include peptides having an "SWFVN" portion (SEQ ID NO:115) or "AQYSWFVN" portion (SEQ ID NO:116) of the sequence. Active analogs of SEQ ID NO:41 include peptides having an "NRQII" portion (SEQ ID NO:199) or "GNRQI" portion (SEQ ID NO:200).

The polypeptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-fluorenylmethoxy-carbonyl (Fmoc) protecting groups. This methodology is described by G.B. Fields et al. in Synthetic Peptides: A User's Guide, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992). The present peptides may also be synthesized via recombinant techniques well known to those skilled in the art. For example, U.S. Patent No. 5,595,887 describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic methods to produce the desired target peptide.

The peptides of the present invention may be employed in a monovalent state (e.g., free peptide or peptide coupled to a carrier molecule or structure).

The peptides may also be employed as conjugates having more than one (same or different) peptide bound to a single carrier molecule. The carrier molecule or structure may be microbeads, liposomes, biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin, or the like), a synthetic polymer

(e.g., a polyalkyleneglycol or a synthetic chromatography support), biomaterial (e.g., a material suitable for implantation into a mammal or for contact with biological fluids as in an extracorporeal device), or other cell. Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary. In addition, as mentioned above, the use of various mixtures and densities of the peptides described herein may allow the production of complexes that have specific binding patterns in terms of preferred ligands.

The polypeptides can be conjugated to other polypeptides using standard methods known to one of skill in the art. Conjugates can be separated from free peptide through the use of gel filtration column chromatography or other methods known in the art.

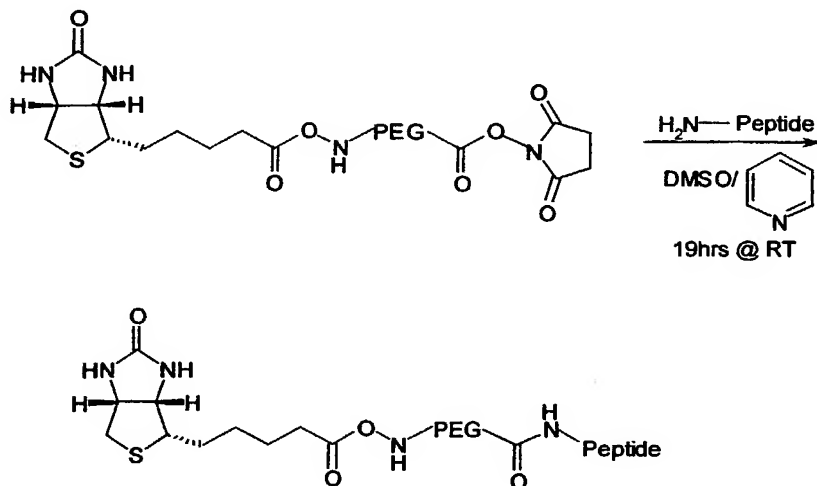
For instance, peptide conjugates may be prepared by treating a mixture of peptides and carrier molecules (or structures) with a coupling agent, such as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule (or structure) so that the carboxyl group can react with a nucleophile (e.g. an amino or hydroxyl group) on the other member of the peptide conjugate, resulting in the covalent linkage of the peptide and the carrier molecule (or structure).

As another example, peptides may be coupled to biotin-labeled polyethylene glycol and then coupled to avidin containing compounds, for instance, as shown in Fig. 13. Peptides are weighed out in aliquots of 0.5 mg and dissolved in a total volume of 500 μ l dimethyl sulfoxide (DMSO, FisherChemical, Fair Lawn, NJ) in a 1 mL ReactiVial containing a flea bar. To each ReactiVial, 1.0 mg Biotin-PEG-NHS, average MW 3400, (Shearwater Polymers, Huntsville, AL) is added directly and the vial is moved to a stir plate to provide gentle mixing. Pyridine (Sigma Chemical, St. Louis, MO) is added as a basic catalyst at a 5% molar excess to the peptide. The reaction is allowed to proceed for 19 hours at room temperature with medium stirring.

After completion of the reaction, the contents of each ReactiVial are individually transferred to a 1.5 mL plastic microfuge tube. Each vial is washed

once with 25 μ l DMSO which is also added to the microfuge tube. The volume of DMSO is dried down at room temperature to approximately 20 μ l of remaining solvent in a Savant Speed Vac Plus. To each tube individually, 980 μ l of Hanks balanced salt solution (HBSS) + 0.1% sodium azide is added.

- 5 Samples are stored at -20°C until coupling to streptavidin-coated beads.



Reaction scheme for biotinylation of peptides.

10

Streptavidin-coated 6 μm diameter polystyrene beads are obtained from Polysciences (Warrington, PA). For each peptide, 100 μ l of suspended beads are aliquoted to a 1.5 ml plastic microfuge tube. As per the manufacturer's directions, the beads are washed three times by sequentially pelleting the beads in a microcentrifuge, decanting the supernatant and redispersing them in 1 ml of fresh phosphate buffered saline (PBS). One third (333 μ l) of the biotinylated peptide from the above preparation is added to the beads in a total volume of 1 ml. From the reported binding capacity of the streptavidin-coated beads, this amount of pegylated peptide represents more than a two-fold molar excess, thus the biotin binding sites are believed to be saturated. The tubes are mixed end-to-end on a rocker plate at 100 revolutions per minute (RPM) for 1 hour. The beads are then washed once as before and resuspended in 1 ml of a 0.1 M ethanolamine solution and mixed on the rocker plate as before for 30 minutes. This step serves to block any potentially unreacted NHS moieties. The beads

15

20

are again washed once as before and resuspended in HBSS + 0.1% sodium azide. In the case of peptides coupled to other entities, it should be understood that the designed activity may depend on which end of the peptide is coupled to the entity.

5 The present invention also provides a composition that includes one or more active agents (i.e., polypeptides) of the invention and one or more pharmaceutically acceptable carriers. One or more polypeptides with demonstrated biological activity can be administered to a patient in an amount alone or together with other active agents and with a pharmaceutically
10 acceptable buffer. The polypeptides can be combined with a variety of physiological acceptable carriers for delivery to a patient including a variety of diluents or excipients known to those of ordinary skill in the art. For example, for parenteral administration, isotonic saline is preferred. For topical administration, a cream, including a carrier such as dimethylsulfoxide (DMSO),
15 or other agents typically found in topical creams that do not block or inhibit activity of the peptide, can be used. Other suitable carriers include, but are not limited to alcohol, phosphate buffered saline, and other balanced salt solutions.

The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.
20 Preferably, such methods include the step of bringing the active agent into association with a carrier that constitutes one or more accessory ingredients.

The methods of the invention include administering to a patient, preferably a mammal, and more preferably a human, the composition of the invention in an amount effective to produce the desired effect.

25 The peptides can be administered as a single dose or in multiple doses. Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art.

30 The agents of the present invention are preferably formulated in pharmaceutical compositions and then, in accordance with the methods of the invention, administered to a patient, such as a human patient, in a variety of forms adapted to the chosen route of administration. The formulations include,

but are not limited to, those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic, or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, intraorgan, intraarterial and intravenous) administration.

Formulations suitable for parenteral administration conveniently include
5 a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Absorption of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

10 Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes containing the active agent, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a
15 syrup, an elixir, an emulsion, or a draught. Such compositions and preparations typically contain at least about 0.1 wt-% of the active agent. The amount of polypeptide (i.e., active agent) is such that the dosage level will be effective to produce the desired result in the patient.

Nasal spray formulations include purified aqueous or other solutions of
20 the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic
25 formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

Examples

Materials and Methods

Cell Preparation. Normal peripheral blood neutrophils were prepared by a modification of the method of Boyum as previously described (64) and were
5 suspended at the indicated concentrations in Hanks' balanced salt solution (HBSS) (Gibco, Grand Island, NY). Differential cell counts on Wright-stained cells routinely revealed greater than 95% neutrophils. Viability as assessed by trypan blue dye exclusion was greater than 98%.

Antibodies and Reagents. The PE-labeled CD11b mAb (Leu 15) and the
10 CD62L mAb (Leu 8) were obtained from Becton Dickinson, Mountain View, CA. Monoclonal antibodies were diluted in PBS containing 1 mg/ml BSA as indicated. N-formyl-met-leu-phe (FMLP) and normal mouse serum (NMS) were purchased from Sigma Chemical Co. (St. Louis, MO). Peptides were diluted in PBS containing 1 mg/ml BSA as indicated.

15 Fluorescence labeling of cells. Neutrophils were labeled with calcein AM (Molecular Probes, Eugene, OR) (65, 66) by incubating 5×10^6 /ml cells with 50 μ g of calcein AM for 30 min at 37°C in 18 ml of calcein labeling buffer (HBSS without Ca^{2+} or Mg^{2+} containing 0.02% BSA). Cells were then washed twice with calcein labeling buffer at 23°C and resuspended in the desired media.

20 Endothelial cell adhesion assay. Neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) was determined as previously described (65-68). Briefly, HUVECs (Clonetics Corp., San Diego, CA) were passaged 1:5 in T-25 flasks (Costar) no more than three times before plating in 96 well microtiter plates at 3000 cells/well. HUVECs were grown to
25 confluence in 96 well microtiter plates in EGM media (Clonetics) and fed every 24 hours. Using the adhesion assay described below, no difference in resting and stimulated neutrophil adhesion was observed, and, as expected (69), no difference in surface expression of CD54 (ICAM-1) or CD62E (E selectin, ELAM-1) in resting or TNF stimulated cells was noted using HUVECs
30 passaged once compared with those passaged five times. In some experiments, the HUVECs were stimulated by culture for the indicated time with the desired cytokines (TNF-alpha (Cetus, Emeryville, CA) or gamma-IFN (gift from Dr. S. Palm, University of Minnesota Medical School)). The wells were then washed

four times with adhesion buffer (DMEM + 5% heat inactivated fetal bovine serum (HIFBS)) and 25 μ l of adhesion buffer containing the indicated peptide was added to each well, followed immediately by 100 μ l of adhesion buffer containing 10^5 calcein labeled cells. Twenty-five microliters of adhesion buffer
 5 containing the indicated concentration of FMLP was then added, and the plates were incubated at 37°C in 5% CO₂ for 30 min. The wells were then aspirated and washed four times with endo wash buffer (HBSS + 4% HIFBS), and the fluorescence was quantitated with a Millipore fluorescence plate reader using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. For
 10 each condition, quadruplicate wells were tested and values are reported as the mean +/- SD. Each experiment was performed at least four times using different HUVEC subcultures.

Statistical analyses. Effects of peptides on neutrophil adhesion to HUVECs was analyzed by the Mann Whitney U test when appropriate.

Analysis of CD11b and CD62L expression. For analysis of CD11b
 15 upregulation, purified neutrophils (10^5 in 100 μ l HBSS + 0.02% BSA) were incubated with media containing the indicated peptide (167 μ g/ml) or FMLP (10^{-7} M) for 15 min at 37°C. The cells were then cooled to 0°C for 10 min and 2 μ g of the PE-labeled CD11b mAb was added. The mixture was incubated at
 20 0°C for 25 min, and 4 ml of buffer B (PBS, pH 7.4, 0.2% BSA, 0.05% NaN₃) (0°C) was then added and the mixture was centrifuged at 400 x g for 5 min at 4°C. The supernatant was removed and the cells were vortexed, and suspended in 1 ml of buffer B (0°C), and 250 ml of fixative (Coulter) (23°C) was then added. Three ml of buffer B (0°C) was then added, and the mixture centrifuged
 25 at 400 x g at 4°C for 5 min. The cells were washed with 3 ml of buffer B as above, and resuspended in 200 ml of PBS containing 0.1% NaN₃ (0°C) and stored at 4°C until analysis. Quantitative flow cytometric analysis of surface antigen expression was performed using a FACSTAR Plus (Beckton Dickinson, Mountain View, CA). Forward and right angle light scatter, as well as the peak
 30 fluorescence channel, were optimized with fluorescent beads. The cell population studied was determined by forward and right angle light scatter.

For analysis of CD62L down regulation, purified neutrophils (10^5 in 100 μ l HBSS + 0.02% BSA) were warmed to 37°C for 5 min and then incubated

with media containing the indicated peptide (167 µg/ml) or FMLP (10^{-7} M) for 5 min at 37°C. The cells were then cooled to 0°C for 10 min and 5 µg of the PE-labeled CD62L mAb was added. The cells were then incubated, washed, and analyzed by flow cytometry as above.

5 Peptide selection, synthesis, and purification. CD66a was modeled to conform to the IgV and Ig C2 domains of the heavy and light chains of Fab fragments of immunoglobulin and CD4.

Peptides were synthesized as amides by Fmoc solid-phase methodology on a Gilson Automated Multiple Peptide Synthesizer AMS 422. Peptides were
10 purified by preparative reverse phase-HPLC on a Beckman System Gold equipped with a Regis Chemical ODS C18 column (10 µm particle size, 60 Angstrom pore size, 250 x 21.1 mm). The elution gradient was 12-50% B over 35 min at a flow rate of 5.0 ml/min, where A is water containing 0.1% trifluoroacetic acid, and B is acetonitrile containing 0.1% trifluoroacetic acid.
15 Detection was at 235 nm. Peptide purity and fidelity can be analyzed by amino acid analysis and sequencing or by mass spectrometry.

Once the first peptides were screened in our adhesion assay (Fig. 1), the amino acids in the positive peptides, CD66a-1, CD66a-2, and CD66a-3, were randomly scrambled and the control peptides were synthesized (Table II). The
20 scrambled amino acid residue peptides were then tested in the same assays in order to ensure that the primary amino acid sequences were essential for the functional activity of these peptides, and that the biological activity was not merely due to the peptides' net charge or amino acid composition (Fig. 3).

Flow cytometry demonstrated that CD66a-1, CD66a-2, and CD66a-3
25 upregulated CD11b and down regulated CD62L neutrophils (Fig. 4).

We next completed the synthesis of peptide CD66a-7 and found that it has similar activity as CD66a-1 (Fig. 5).

Since peptide CD66a-6 was not soluble we synthesized peptides from the same region but shifted the center of the peptide in an attempt to generate a
30 soluble peptide. One peptide, CD66a-6L, (Table I) was successfully synthesized, tested, and found to stimulate neutrophil adhesion to HUVECs (Fig. 6).

Since only the N-domain peptide of CD66a had activity in the neutrophil activation assay we modeled CD66b, CD66c, CD66d, CD66e, and CD66f N-domains and synthesized appropriate peptides as shown in Tables III-VIII. Of these peptides, only peptide CD66e-3 activated neutrophils (Fig. 7). These results are noteworthy in that many peptides that have only minor differences from active peptides had no biological activity.

Method #1 for adhesion assay of CHO transfectant binding to immobilized recombinant CD66a (Assay #1). Stable CHO cell transfectants expressing CD66a (CEACAM1-4L) CEACAM1-4S, CEACAM1-1S or the neomycin resistance gene (CHO-Neo) (provided by Dr. S. Watt, MRC, Oxford, UK) were grown to 50-70% confluence in Hams-F10 medium containing 10% (v/v) FBS. Adherent cells were detached with PBS containing 1 mM EDTA pH 7.4, washed three times with Hams-F10 medium, and resuspended in Hams-F10 medium at 2×10^6 cells/ml. The fluorescent dye 2',7'-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluoresceinacetoxymethylester (BCECF-AM); Molecular Probes, Eugene, OR) was dissolved in DMSO at 500 $\mu\text{g/ml}$ and 20 μl added per 2×10^7 cells for 20-30 min at 37°C. Cells were washed twice with RPMI-1640 medium and twice with PBS containing 0.2% BSA (PBS-0.2% BSA). Cells (5×10^4) in PBS-0.2% BSA were added to 96 well Immulon 3 flat bottomed microtiter plates (Dynatech) that had been pre-coated as follows. Purified goat anti-human Fc antibody (Sigma Chemical Co.) was added to 96 well flat bottomed Immulon 3 plates at 1 $\mu\text{g}/100 \mu\text{l/well}$ at 4°C overnight. The plates were washed 4 times with PBS containing 0.5% BSA (PBS-0.5% BSA) and blocked with PBS-0.5% BSA for at least two hours at room temperature. After washing the plates 4 times with PBS, 50 μl of soluble recombinant protein containing the Fc fragment of human IgG1 attached to CEACAM1 (CEACAM1-Fc) or other indicated protein (10 $\mu\text{g/ml}$) in PBS were added for at least 2 hours at room temperature or overnight at 4°C. The plates were washed 4 times with PBS before the addition of 100 μl of cells. CHO cell transfectants labeled with BCECF-AM were allowed to adhere for 60 min at 37°C before reading the total BCECF-AM fluorescence in each well on a Cytofluor II plate reader (PerSeptive Biosystems, Hertford, UK) at an excitation wavelength of

485/20 nm, a gain of 70 and an emission wavelength of 530/30 nm. The plates were washed one to three times with PBS-0.2% BSA and the percentage of cells adhering to the constructs estimated from the subsequent fluorescence determinations on the Cytofluor II. Adhesion assays were performed with 4 to 5 6 replicates in at least two independent experiments.

Method #2 for adhesion assay of CHO transfectant binding to immobilized recombinant CD66a (Assay #2). CHO cells transfected with BGPα cDNA (courtesy Dr. M. Kuroki) were grown in α-MEM (Gibco Inc., Grand Island, NY) lacking nucleosides with 10% FBS (Bio-Whittaker, Walkersville, MD) and antibiotics. Cell cultures were maintained by passing 1:10 in T-25 flasks approximately every 3 days. For the assay, one T-25 of cells near confluence was trypsinized and the collected population was washed once with growth media and resuspended 0.5 mL growth media. To obtain a single-cell suspension, cells were passed sequentially through an 18-gauge, 22-gauge and 25-gauge needle. 10 15

One μg of protein in 50 μl of PBS was dried down in a well of a 96-well plate. Wells were incubated with 0.5% BSA in PBS (200 μl/well) for blocking for 4 hours at room temperature. CHO transfectants expressing the appropriate CD66 family member were incubated in serum-free MEM containing 25 μg/ml H33342 dye at 1×10^6 /ml for 30 min at 37°C (other dyes such as calcein can be used). After washing, cells were suspended in PBS at 1×10^6 /ml. PBS (35 μl) and 15 μl (15 μg) of peptide solution was added into a protein-coated well, and then 5×10^4 cells (50 μl) labeled with dye were added. After vortexing gently, 20 25 the plate was incubated at room temperature for 25 min in the dark. Each well was gently washed with 100 μl PBS twice. Remaining cells were solubilized in 100 μl/well of PBS containing 0.2% NP40 and fluorescence was measured by a microplate reader.

The data is shown as percent of added cells that remained adherent. Control values "0" and "1000" represent the adhesion observed when no CD66α protein is adherent to the well, or when buffer is added without peptide, respectively. Control values "0.01" represent the adhesion observed in the presence of a blocking CD66 antibody. 30

Assay for binding of peptides coupled to beads to CHO cells expressing recombinant CD66a. CHO cells transfected with BGPa cDNA were grown and prepared as in Assay #2. To each tube containing 10 µl peptide-bound beads (approximately 300,000 beads) 20 µl of cell suspension was added and mixed gently. The tubes were then incubated for 30 minutes at room temperature.

After incubation, aliquots were taken from each tube and placed on a glass slide. For each sample, data was quantified by viewing five separate fields under a scope at 125x magnification and counting the number of beads associated with single cells or groupings of cells in three size classes.

Example 1 – Effect of peptides on neutrophil activation determined by adhesion to endothelial cells

The CD66a peptides were tested for their ability to alter neutrophil adhesion to human umbilical vein endothelial cells (HUVECs) stimulated for 48 hours with 1000 U/ml gamma-interferon and 50 ng/ml TNF-alpha (Fig. 1). When neutrophils were incubated for 30 min in the presence of media containing 167 µg/ml of each peptide with these HUVECs, and washed as described in the Endothelial Adhesion Assay, three peptides (CD66a peptides CD66a-1, CD66a-2, and CD66a-3) augmented neutrophil adhesion approximately two-fold compared with media (Fig. 1, solid bars). This effect was more prominent in the presence of 10^{-7} M FMLP (hatched bars). In contrast, the other peptides did not alter neutrophil adhesion when compared with incubation in media alone. Similar results were obtained using HUVECs stimulated for 4 hours with 50 µg/ml TNF-alpha (not shown).

The three peptides that specifically induced neutrophil adhesion were further tested for their effects on the adhesion of neutrophils to TNF stimulated HUVECs. Each of the three CD66a peptides, CD66a-1, CD66a-2, and CD66a-3, increased neutrophil adhesion to HUVECs at concentrations as low as 50 µg/ml (approximately 35 µM) in the presence of FMLP (Fig. 2). To confirm that the activity of these peptides was due to the sequence and not simply a charge effect, three scrambled versions were made of each active peptide (Table

II) and tested in the adhesion assay. In contrast to the native peptides, none of the 9 scrambled peptides had activity in the adhesion assay (Fig. 3).

Example 2 – Effect of peptides on neutrophil activation determined by
5 expression of surface CD11b and CD62L

Effect of CD66a peptides on CD11b expression. The effects of the peptides on surface expression of CD11b on neutrophils was next examined. While neutrophil adhesion to HUVECs is dependent on the functional activity of surface CD11/CD18, many adhesive stimuli also upregulate the surface
10 expression of CD11/CD18, and this may play a role in regulating cell adhesion as well (70-72). To determine if an alteration in the surface expression of CD11/CD18 could contribute to the effect of the CD66a peptides on neutrophil adhesion, CD11b expression was analyzed by flow cytometry. Since CD11 and CD18 are translocated to the cell surface only when they are complexed with
15 each other, the use of a directly labeled CD11b mAb was used to demonstrate upregulation of CD18 as well as CD11b. When neutrophils were incubated with HBSS for 15 min at 37°C and then reacted with a PE-labeled CD11b mAb, CD11b expression was readily detected by flow cytometry (MCF = 584) (Fig. 4, top panel). As expected, when neutrophils were incubated with FMLP (10^{-7}
20 M) for 15 min, CD11b expression was increased (MCF = 709) (second panel). When neutrophils were incubated with 167 µg/ml of the CD66a peptide CD66a-1 (MCF = 704) (third panel), the CD66a peptide CD66a-2 (MCF = 713) (fourth panel), or the CD66a peptide CD66a-3 (MCF = 714) (fifth panel), CD11b expression also increased, similar to that seen with incubation with 10^{-7} M
25 FMLP. In contrast, incubation with the scrambled CD66a peptide CD66a-1-S1 resulted in similar CD11b expression as incubation with HBSS (MCF = 581) (bottom panel), as did the other eight scrambled peptides (not shown).

Effect of CD66a peptides on CD62L expression. The effects of the peptides on surface expression of CD62L on neutrophils was next examined. L-selectin, recognized by CD62L mAbs, also plays a role in neutrophil adhesion
30 to endothelial cells, and its expression is altered by stimulation (70, 72). To determine if the surface expression of CD62L could be altered by CD66a peptides, CD62L expression was analyzed by flow cytometry. When

neutrophils were incubated with HBSS for 5 min at 37°C, and then reacted with a PE-labeled CD62L mAb, CD62L expression was readily detected by flow cytometry (MCF = 548) (Fig. 4, top panel). When neutrophils were incubated with 10^{-7} M FMLP, CD62L expression decreased as expected (MCF = 256) (second panel). Similarly, when neutrophils were incubated with the CD66a peptide CD66a-1, (MCF = 230) (third panel), the CD66a peptide CD66a-2 (MCF = 243) (fourth panel), or the CD66a peptide CD66a-3 (MCF = 229) (fifth panel), CD62L expression also decreased. Incubation with the scrambled CD66a peptide CD66a-1-S1 did not alter CD62L expression (MCF = 546) (bottom panel). Similarly, none of the other eight scrambled peptides altered CD62L expression (not shown).

As described above, three other peptides from the N-domains of CD66a, b, c, d, and e, but no other N-domain peptides, were also found to activate neutrophil adhesion to HUVECs (Figs. 5-7).

Example 3 - Modulation of binding of CHO cells expressing recombinant CD66 family members to recombinant CD66 family member proteins in solid phase binding assay #1

Homotypic and heterotypic adhesion was assayed using two different techniques. In Assay #1 the adhesion of CHO cells expressing recombinant CD66a to recombinant CD66a-Fc bound to anti-Fc immobilized to a microtiter well was quantitated in the presence and absence of peptides. Four peptides were found to block CD66a-CD66a adhesion in this assay: CD66a-17; CD66a-18; CD66a-19; and CD66a-24 (Fig. 8).

In Assay #2 the adhesion of CHO cells expressing the appropriate CD66 family member to the desired recombinant CD66 family member immobilized to a microtiter well was quantitated in the presence and absence of peptides (Figs. 9-12).

Example 4 - Binding of microbeads coupled to peptide CD66a-24 to CHO transfectants expressing CD66a

One application of these peptides is their use to target binding of larger structures to specific cells/tissues. The complexing of one or more of the

described peptides to a larger entity should result in binding of the complex to cells expressing the appropriate ligands (for example, CD66a in tumors or CD66a in growing endothelial cells involved in angiogenesis).

CD66a-24 and CD66a-1 peptides were coupled to microbeads and the microbeads were incubated with a suspension of CHO cells expressing CD66a at room temperature for 30 min. The binding of the microbeads to the CHO cells was quantified by counting the number of beads associated with single cells or groups of cells in three cell-group size classes and are reported as the number of microbeads bound to each size group of cells (Fig. 13A). Fig. 13B shows the number of beads associated with single cells, which are reported as the average number of microbeads bound to each single cell. The lack of binding of CD66a-1 coupled beads serves as a negative control for this experiment but does not imply that a different coupling technique would not result in binding.

Example 5 -- Effects of peptides on T-cell activation

Cytotoxic lymphocytes are felt to play a key role in the immune response to malignant transformation. T-cells play an important role in the immune system, and a number of cell-surface molecules have been found to regulate T-cell activation (88, 90, 91, 92). Thus, we tested the effects of CD66 peptides on T-cell activation as determined by proliferation following stimulation by anti-CD3.

Blood lymphocytes were stimulated by anti-CD3 in vitro in the presence of the indicated peptides and proliferation was determined by radioactive nucleotide incorporation. The data are reported as cpm +/- SD. Biological activity was detected in this assay for peptides: CD66a-24 and CD66e-29 (Fig. 14).

Discussion

Peptides were synthesized from regions of CD66 family members that we predict may be exposed on the surface of the molecule. Three of the peptides were found to have activity in an assay examining stimulated neutrophil adhesion to HUVECs. These same three peptides also stimulated

upregulation of CD11b/CD18 and down regulation of CD62L on the neutrophil surface. Scrambled versions of these peptides had no biological activity in either assay, suggesting that the specific amino acid sequence is critical for activity. Thus, the data suggest that peptide motifs from at least three regions of the N-terminal domain of CD66a are involved in the interaction of CD66a with other ligands and can initiate signal transduction in neutrophils. Three other peptides from CD66 family members also stimulated neutrophils.

Several other studies have proposed structural motifs of CD66a family proteins (16, 21, 73).

All neutrophil activating peptides identified in this study are derived from the N-terminal domains of CD66a or CD66e. Studies of transfectants and recombinant proteins have suggested that the N-terminal domain is critical for the homotypic and heterotypic adhesion activity of CD66a (12, 21, 23, 25, 32).. Studies using domain specific mAbs have also suggested that the N-domains of CD66 family members are important in homotypic adhesion (21, 24).. However, studies have also suggested that the A1, B1, or A2 domains may also be important in homotypic adhesion, and may interact with the N-domain (12, 19, 20, 22, 23).

Although carbohydrates on CD66 family members may play important roles, the protein backbone itself appears to have important activity in this and other studies. For example, bacterial fusion proteins free of carbohydrates containing the N or A3B3 domains of CD66e can block CD66e homotypic adhesion, demonstrating that protein-protein interaction is involved in CD66e homotypic adhesion (23). Deglycosylated forms of CD66b and CD66c retain heterotypic adhesion activity (31), further demonstrating that carbohydrates are not necessary for their adhesion functions. In addition, both recombinant N-terminal domains of CD66a and CD66e expressed in *Escherichia coli* bind Opa proteins with the same specificities as native CD66 molecules, and deglycosylated forms of CD66e bind bacterial Opa proteins (50).

Site directed mutagenesis studies of the related proteins C-CAM-105 and CEA (CD66e) have identified regions important for certain functional activities. For example, the integrity of Arg-98 in the consensus ATPase domain (GPAYSGRET) of C-CAM-105 is essential for homotypic aggregation

(58). This arginine is highly conserved in Ig domains, being important in forming a salt bridge with a highly conserved aspartate within the same domain (16). In our model the consensus ATPase domain is present in the sequence of peptide CD66a-5. However, peptide CD66a-5 had no activity in our assay.

5 The finding that these short peptides can stimulate neutrophils, as can CD66a mAbs (26-28, 67, 74, 75) suggests that they have significant affinity for a surface structure, possibly native CD66a. If so, whether the activity derives from binding native CD66a and transducing a signal directly, or by another mechanism will require further study. The ability of the synthetic peptides
10 described here to activate neutrophils could be mediated by alterations in CD66a dimerization, possibly by disrupting a preexisting association of CD66a with other CD66 members (including CD66a itself in the form of dimers or oligomers already present on the cell surface) or by stimulating dimerization. It has been suggested that CD66a (76) and CD66e (77) exist on the cell surface as
15 dimers. Dimerization of CD66a could potentially occur via interactions between the extracellular domains of CD66a molecules or via other mechanisms. In other receptor systems (e.g. EGF-monomeric, PDGF-dimeric), it is clear that bivalency of ligand is not necessary to induce receptor dimerization (78-81). Finally, the observed functional "stimulation" could
20 reflect either "stimulation" per se or possibly release from a baseline inhibition.

 The mechanisms by which CD66 family members transmit signals (e.g. activation in neutrophils, immune suppression of T-lymphocytes, or growth regulating signals in epithelial cells and carcinomas) are unclear. CD66a is phosphorylated in neutrophils and colon cancer cells (4, 59-61), and associated
25 protein kinase and phosphatase activity may be involved (59, 62). At least eight isoforms of CD66a derived from differential splicing have been described (3, 12, 13, 25). These isoforms contain one N-domain, either three, two, or no Ig C2-like domains, and either a short or a long cytoplasmic tail. Only those
30 isoforms with a long cytoplasmic tail can be phosphorylated on tyrosine, and only the isoform with four Ig domains and a long cytoplasmic tail (the only isoform detected in neutrophils) have been implicated in signaling. The cytoplasmic domain of neutrophil CD66a contains an immune tyrosine inhibitory motif (ITIM), as well as a motif similar to ITAM (immune tyrosine

activating motif) (3, 59). Phosphorylation of ITAMs and ITIMs leads to binding of protein tyrosine kinases and protein tyrosine phosphatases, respectively, which leads to modification of signal transduction (62, 63).

Calmodulin has also been found to bind to the cytoplasmic domain of CD66a, causing an inhibition of homotypic self-association of CD66a in a dot-blot assay (82). CD66a has also recently been shown to dimerize in solution, and calcium-activated calmodulin caused dissociation of CD66a dimers in vitro; suggesting that CD66a dimerization is regulated by calmodulin and intracellular calcium (76). It has been suggested that CD66a dimerization could also be influenced by phosphorylation; CD66a is phosphorylated on Thr-453 in the calmodulin binding site by protein kinase C (3). Clearly, dimerization of CD66a could affect binding of other signal regulating molecules.

CD66 family members appear to be involved in a wide variety of important biological processes, and their differential expression provides the possibility for diverse interactions. For example, CD66a, CD66b, CD66c, and CD66d, but not CD66e, are expressed on neutrophils; CD66e is expressed on many tumor cells but not leukocytes; CD66b is expressed on neutrophils but not epithelial cells; CD66c is expressed on both neutrophils and epithelial cells (reviewed in (1) and (13)). While CD66a was originally described in biliary canaliculi, it has since been found in carcinomas as well as normal tissues, including: sebaceous glands (83, 84), neutrophils, placenta, stomach, breast, pancreas, thyroid, prostate, lung, kidney, uterus, and colon (reviewed in (1) and (25)). The surface expression of these molecules in other cells may also be regulated; for example, CD66a expression is induced on HUVECs following treatment with gamma-IFN (10). In addition, surface expression of CD66 family members may be regulated by other stimuli and this may modify the signal transduction capabilities of cell surface CD66 molecules. Finally, studies have shown that certain bacteria bind to some CD66 family members on neutrophils (45-50, 85, 86) and this interaction may also result in signal transduction resulting in modification of neutrophil activity. The major receptor for murine hepatitis virus is a murine CD66a equivalent (51) (52-55) and studies suggest that this virus uses different murine CD66 family members as the major receptor in different tissues (55). A recent consensus was reached that will

rename the CD66 antigens as follows: CD66a antigen, CEACAM-1; CD66b antigen, CEACAM-8; CD66c antigen, CEACAM-6; CD66d antigen, CEACAM-3, CD66e antigen, CEA (14).

CD66 members appear to play an important role in inflammation. Each of the CD66 family members expressed on neutrophils, CD66a, CD66b, CD66c, and CD66d, are capable of transmitting activation signals in neutrophils, and neutrophil CD66a and CD66c appear to be able to present CD15s (a ligand for ELAM-1 or E-selectin) to E-selectin on endothelial cells in a functional way (26). Recent studies have demonstrated the presence of CD66a on T-lymphocytes and a subset of NK cells (CD16-, CD56+) that predominate in decidua (87), and CD66a is upregulated in activated T-cells (87). Finally, CD66e expression by tumor cells is correlated with resistance to NK/LAK cell mediated lysis (88, 89). Thus, these data suggest that soluble CD66 family members could contribute to the immunosuppression often found in patients with cancer.

The biological activity of the peptides identified here suggests that they may have sufficient affinity to make them potential candidates for drug localization to cells expressing the appropriate surface structures.

Table 1: CD66a Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66a-1	SMPFNVAEGKEVL		1	Incr PMN Adhesion to HUVECs
CD66a-2	LVHNLPPQLFGYSW		2	Incr PMN Adhesion to HUVECs
CD66a-3	KGERVDGNRQIVGY		3	Incr PMN Adhesion to HUVECs
CD66a-4	VGYAIGTQQAATPG		117	
CD66a-5	ATPGPANSGRETIY		118	
CD66a-6	LLIQNVNTQNDTGFY	CD66c-6	119	
CD66a-7	VIKSDLVNEEATGQ	CD66c-7 CD66d-7 CD66e-7	4	Incr PMN Adhesion to HUVECs
CD66a-8	EATGQFHVYPPEPK	CD66c-8 Contains CD66d-8	120	
CD66a-9	NNSNPVEDKDAVAF	CD66b-9 CD66c-9	121	
CD66a-10	PETQDTTYLWWNN		5	Homolog CD66b-10, CD66c-10
CD66a-11	NNQSLPVSPLQLS	CD66e-12 CD66e-27	122	
CD66a-12	LQLSNGNRITLTLIS	CD66b-12	6	Homolog CD66c-12
CD66a-13	TLLSVTRNDTGPYE		123	
CD66a-14	IQNPVSANRSDPVT		124	

CD66a-15	SDPVTILNVTYGPD	CD66b-15 CD66c-15	7	Incr CD66a-CD66a Adhesion (Transflectant Binding Assay #2)
CD66a-16	PSDTYYRPGANLSL		8	Decr CD66a-CD66a Adhesion (Transflectant Binding Assay #2)
CD66a-17	AASNPPAQYSWLIN		9	Decr CD66a-CD66a Adhesion (Transflectant Binding Assays #1 and #2)
CD66a-18	LINGTFQQSTQELF		10	Decr CD66a-CD66a Adhesion (Transflectant Binding Assay #1)
CD66a-19	FIPNITVNNSGSYT	CD66e-21	11	Decr CD66a-CD66a Adhesion (Transflectant Binding Assay #1)
CD66a-20	ANNSVTGCNRTTVK		125	
CD66a-21	TTVKTIIVTELSV		12	Incr CD66a-CD66a Adhesion (Transflectant Binding Assay #2)
CD66a-22	ELSPVVAKPQIKAS		126	
CD66a-23	SKTTVTGDKDSVNL		13	Incr CD66a-CD66a Adhesion (Transflectant Binding Assay #2)
CD66a-24	TNDTGISIRWFFKN		14	Decr CD66a-CD66a Adhesion (Transflectant Binding Assay #1)
CD66a-25	KNQSLPSSERMKLS		127	

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CD66a-26	ERMKLSQGNITLSI	15	Incr CD66a-CD66a Adhesion (Transflectant Binding Assay #2)
CD66a-27	LSINPVKREDAGTY	128	
CD66a-28	FNPIKKNQSDPIM	129	
CD66a-29	ISKKNQSDPIMLVN	16	Homolog CD66e-31
CD66a-5L	GTQQATPGPANSGR	130	
CD66a-5R	SGRETIYPNASLLI	131	
CD66a-6L	TIYPNASLLIQNVVT	17	Incr PMN Adhesion to HUVECs; Decr CD66a-CD66a Adhesion Decr CD66b-CD66c Adhesion (Transflectant Binding Assay #2)

Table II: Scrambled Versions of CD66a Peptides

Peptide Name	Peptide Sequence	SEQ ID NO:	Function
CD66a-1	SMPFNV AEGKEVL	1	Incr PMN Adhesion to HUVECs
CD66a-1-S1	LEFKVEMAPSNVG	132	
CD66a-1-S2	PNVELEFGMKAVS	133	
CD66a-1-S3	ENMPLSAFEVVKG	134	
CD66a-2	LVHNL PQQLFGYSW	2	Incr PMN Adhesion to HUVECs
CD66a-2-S1	QNLLSHLGFVWPQY	135	
CD66a-2-S2	HVQSFLLPNLYQG	136	
CD66a-2-S3	SVLPLGQWHQYNFL	137	
CD66a-3	KGERVDGNRQIVGY	3	Incr PMN Adhesion to HUVECs
CD66a-3-S1	VENQGVGGKRIRDY	138	
CD66a-3-S2	GRYDQNKVIEVRGG	139	
CD66a-3-S3	GIVEYKGV DQNRNG	140	

Table III: CD66b Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66b-1	AVPSNAAEQKEVL		18	Homolog CD66a-1
CD66b-2	LVHNLQDPRGYNW		19	Homolog CD66a-2, CD66e-2
CD66b-3	KGETVDANRRIGY		20	Homolog CD66a-3
CD66b-4	IGYVISNQIITPG		141	
CD66b-5	ITPGPAYSNRETIY		142	
CD66b-6	LLMRNVTKNDTGSY		143	
CD66b-7	VIKLNLMSEEVGTQ		21	Homolog CD66a-7
CD66b-8	EVTGQFSVHPETPK		144	
CD66b-9	NNSNPVEDKDVAFA	CD66a-9 CD66c-9	121	
CD66b-10	PETQNTTYLWWVNG		22	Deer CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66b-11	NGQSLPVSPRLQLS	CD66c-11 CD66e-43	145	
CD66b-12	LQLSNGNRTLILLS	CD66a-12	6	Homolog CD66c-12
CD66b-13	TLLSVTRNDVGPYE	CD66e-29	146	
CD66b-14	IQNPASANFSDPVT		147	

CD66b-15	SDPVTLNVITYGPD	CD66a-15 CD66c-15	7	Incr CD66a-CD66a Adhesion
CD66b-16	PSDTYYHAGVNLNL		23	Homolog CD66a-16
CD66b-17	AASNPPSQYSWSVN		24	Homolog CD66a-17, CD66c-17, CD66e-19
CD66b-18	SVNGTFQQYTQKLF		25	Homolog CD66a-18
CD66b-19	IPNITTKNSGSA		26	Homolog CD66a-19, CD66c-19
CD66b-20	TTNSATGRNRTTVR		148	
CD66b-21	TTVRMITVSDALVQ		27	Homolog CD66a-21
CD66b-5L	SNQITPGPAYSNR	Shift CD66b-5 to left	149	
CD66b-6L	TIYPNASLLMRNVT	Shift CD66b-6 to left	28	Homolog CD66a-6L

Table IV: CD66c Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66c-1	STPFNVAEGKEVL	CD66d-1 CD66e-1	29	Homolog CD66a-1
CD66c-2	LAHNLQNRIGYSW		30	Homolog CD66a-2, CD66e-2
CD66c-3	KGERVDGNSLIVGY	CD66d-3	31	Homolog CD66a-3
CD66c-4	VGYYVIGTQQATPG	CD66d-4	150	
CD66c-5	ATGPAYSGRETTY		151	
CD66c-6	LLIQNVNQNDTGIFY	CD66a-6	119	
CD66c-7	VIKSDLVNEEATGQ	CD66a-7 CD66d-7 CD66e-7	4	Incr PMN Adhesion to HUVECs
CD66c-8	EATGQFHVYPKLPK	CD66a-8 Contains CD66d-8	120	
CD66c-9	NNSNPVEDKDVAFA	CD66a-9 CD66b-9	121	
CD66c-10	PEVQNNTTYLWWVNG		32	Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66c-11	NGQSLPVSPRLQLS	CD66b-11 CD66e-43	145	
CD66c-12	LQLSNGNMILTLLS		33	Decr CD66b-CD66c Adhesion (Transfectant Binding Assay #2)
CD66c-13	TLLSVKRNDAAGSYE		152	

CD66c-14	IQNPASANRSDPVT		153	
CD66c-15	SDPVTILNVTYGPD	CD66a-15 CD66b-15	7	Incr CD66a-CD66a Adhesion (Transflectant Binding Assay #2)
CD66c-16	PSKANYRPGENLNL		34	Homolog CD66a-16
CD66c-17	AASNPPAQYSWFIN		35	Decr CD66b-CD66c Adhesion Decr CD66c-CD66c Adhesion Decr CD66e-CD66e Adhesion (Transflectant Binding Assay #2)
CD66c-18	FINGTFQQSTQELF		36	Homolog CD66a-18
CD66c-19	IPNITVNNSGSYM		37	Decr CD66b-CD66c Adhesion (Transflectant Binding Assay #2)
CD66c-20	AHNSATGLNRITVT		154	
CD66c-21	TTVTMITVSGSAPV		38	Homolog CD66a-21
CD66c-5L	GTQQATPGPAYSGR	CD66c-5L Shift CD66c-5 to left	155	
CD66c-6L	TYYPNASLLIQNVIT	CD66a-6L Shift CD66c-6 to left	17	Incr PMN Adhesion to HUVECs Decr CD66a-CD66a Adhesion Decr CD66b-CD66c Adhesion (Transflectant Binding Assay #2)

Table V: CD66d Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66d-1	STPFNVAEGKEVL	CD66c-1 CD66e-1	29	Homolog CD66a-1
CD66d-2	LVHNL PQH LFGYSW	CD66e-2	39	Decr CD66e-CD66e Adhesion (Transflectant Binding Assay #2) Homolog CD66a-2, CD66e-2
CD66d-3	KGERVDGNSLIVGY	CD66c-3	31	Homolog CD66a-3
CD66d-4	VGYYIGTQQATPG	CD66c-4	150	
CD66d-5	ATPGAAYSGRETIY		156	
CD66d-6	LLIHNVITQNDIGFY		157	
CD66d-7	VIKSDLVNEEATGQ	CD66a-7 CD66c-7 CD66e-7	4	Incr PMN Adhesion to HUVECs
CD66d-8	EATGQFHVY	Part of CD66a-8 and CD66c-8	158	
CD66d-5L	GTQQATPGAAYSGR	Shift CD66d-5 to left	175	
CD66d-6L	TIYTNASLLIQNVT	Shift CD66d-6 to left	40	Homolog CD66a-6L

Table VI: CD66e Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66e-1	STPFNVAEGKEVL	CD66c-1 CD66d-1	29	Homolog CD66a-1
CD66e-2	LVHNLPPQLFGYSW	CD66d-2	39	Decr CD66e-CD66e Adhesion (Transflectant Binding Assay #2)
CD66e-3	KGERVDGNRQIIIGY		41	Incr PMN Adhesion to HUVECs
CD66e-4	IGYVIGTQQATPG		159	
CD66e-5	ATPGPAYSGREIYY		160	
CD66e-6	LLIQNIQNNDTGFY		161	
CD66e-7	VKSDLVNEEATGQ	CD66a-7 CD66c-7 CD66d-7	4	Incr PMN Adhesion to HUVECs
CD66e-8	EATGQFRVYPELPK		162	
CD66e-9	YPELPKPSISSNNS		163	
CD66e-10	NNSKPVEDKDAVAF	CD66e-41	164	
CD66e-11	PETQDATYLVWVNN		42	Homolog CD66b-10, CD66c-10
CD66e-12	NNQSLPVSPLQLS	CD66a-11 CD66e-27	122	
CD66e-13	LQLSNGNRRTLTLFN		43	Homolog CD66c-12
CD66e-14	TLFNVTRNDTASYK		165	

CD66e-15	TQNPVSARRSDSVI		166		
CD66e-16	SDSVILNVLYGPDA		44		Homolog CD66a-15
CD66e-17	NVLYGPDAPTISPL		45		Homolog CD66a-15
CD66e-18	PLNTSYRSGENLNL		46		Homolog CD66a-16
CD66e-19	AASNPPAQYSWFVN		47		Decr CD66b-CD66c Adhesion Decr CD66c-CD66c Adhesion Decr CD66e-CD66e Adhesion (Transflectant Binding Assay #2)
CD66e-20	FVNGTFQQSTQELF		48		Homolog CD66a-18
CD66e-21	FIPNITVNNSGSYT	CD66a-19	11		Decr CD66a-CD66a Adhesion (Transflectant Binding Assay #1)
CD66e-22	AHNSDTGLNRITVT		167		
CD66e-23	TTVTITIVYAEPPK		49		Homolog CD66a-21
CD66e-24	TVYAEPPKPFITSN		168		
CD66e-25	NNSNPVEDEDAVAL		50		Homolog CD66a-23
CD66e-26	PEIQNTTYLWWVNN		51		Homolog CD66a-24
CD66e-27	NNQSLPVSPRLQLS	CD66a-11 CD66e-12	122		
CD66e-28	LQLSNDNRTLTLSS		52		Homolog CD66a-26
CD66e-29	TLLSVTRNDVGPYE	CD66b-13	146		
CD66e-30	IQNELSVDHSDPVI		169		

CD66e-31	SVDHSDPVILNVLY		53	Decr CD66e-CD66e Adhesion (Transfected Binding Assay #2)
CD66e-32	SDPVILNVLYGPDD		85	
CD66e-33	NVLYGPDDPTISPS		86	
CD66e-34	PSYTYRPGVNLSL		87	
CD66e-35	AASNPPAQYSWLID		88	
CD66e-36	LIDGNIQQHTQELF		89	
CD66e-37	ISNITEKNSGLYT		90	
CD66e-38	ANNSASGHSRTTVK		170	
CD66e-39	TTVKTITVSAELPK		91	
CD66e-40	TVSAELPKPSISSN		171	
CD66e-41	NNSKPVEDKDAVAF	CD66e-10	164	

CD66e-42	PEAQNTTYLWWVNG		54	Decr CD66e-CD66e Adhesion (Transflectant Binding Assay #2)
CD66e-43	NGQSLPVSRLQLS	CD66b-11 CD66c-11	145	
CD66e-44	LQLSNGNRTLTLFN		92	
CD66e-45	TLFNVTRNDARAYV		172	
CD66e-46	IQNSVSANRSDPVT		173	
CD66e-47	SANRSDPVTLDVLY		93	
CD66e-48	SDPVTLDVLYGPD		94	
CD66e-49	DVLYGPDTPHIPP		95	
CD66e-50	PPDSSYLSGANLNL		96	
CD66e-51	SASNPSQPYSWRIN		97	
CD66e-52	RINGIPQQHTQVLF		98	
CD66e-53	IAKITPNNNGTYA		99	
CD66e-54	VSNLATGRNNSIVK		174	
CD66e-55	NNSIVKSITVSASG		100	

CD66e-5L	GTQQAATPGPAYSGR	CD66c-5L Shift CD66e-5 to left	155		S174
CD66e-6L	IYPNASLLIQNI	Shift CD66e-6 to left	55	Homolog CD66a-6L	S177

Table VII: CD66f(1) Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66f(1)-1	AQPPKVSEKDV L		56	Homolog CD66a-1
CD66f(1)-2	LVHNL PQNL TGYIW		57	Homolog CD66a-2, CD66e-2
CD66f(1)-3	KGQMRDLYHYT SY		58	Homolog CD66a-3
CD66f(1)-4	TSYVV DGEIIIYG		176	
CD66f(1)-5	IYGPAYSGRETAY		177	
CD66f(1)-6	LLIQNV TREDAGSY		178	
CD66f(1)-7	IIKGDDGTRGV TGR		59	Homolog CD66a-7
CD66f(1)-8	GVTGRFTILHLETPK		179	
CD66f(1)-9	NNLNPRENKDVLNF		180	
CD66f(1)-10	PKSENYTYIWWLNG		60	Homolog CD66b-10, CD66c-10
CD66f(1)-11	NGQSLPVSPRVKRP		181	
CD66f(1)-12	VKRPIENRILILPS		61	Homolog CD66c-12
CD66f(1)-13	ILPSVTRNETGPYQ		182	
CD66f(1)-14	IRDRYGGVRS DPVT		183	
CD66f(1)-15	SDPVT LNVLYGPD L		62	Homolog CD66a-15

CD66f(1)-16	PSFTYYRSGEVLYL		63	Homolog CD66a-16
CD66f(1)-17	ADSNPPAQYSWTIN		64	Homolog CD66a-17
CD66f(1)-18	TINEKFQLPGQKLF		65	Homolog CD66a-18
CD66f(1)-19	IRHITTKHSGLYV		66	Homolog CD66a-19, CD66c-19
CD66f(1)-20	VRNSATGKESKSM		184	
CD66f(1)-21	SKSMTVEVSEAL		67	Homolog CD66a-21

Table VIII: CD66f(11) Peptides

Peptide Name	Peptide Sequence	Identical to Peptides from other CD66 Families	SEQ ID NO:	Function and/or Homology to Functional Peptide
CD66f(11)-1	AQPPKVSEKDVLL		68	Homolog CD66a-1
CD66f(11)-2	LVHNLQNLPGYFW		69	Homolog CD66a-2, CD66e-2
CD66f(11)-3	KGEMTDLYHYTISY		70	Homolog CD66a-3
CD66f(11)-4	ISYIVDGKIIIVG		185	
CD66f(11)-5	IYGPAYSGRETVY		186	
CD66f(11)-6	LLIQNVTRKDAGTY		187	
CD66f(11)-7	IKRGDETREEIRH		71	Homolog CD66a-7
CD66f(11)-8	EEIRHFTFTLYLETPK		188	
CD66f(11)-9	SNLNPREAMEAVRL		189	
CD66f(11)-10	PETLDASYLWWMNG		72	Homolog CD66b-10, CD66c-10
CD66f(11)-11	NGQSLPVTHRLQLS		190	
CD66f(11)-12	LQLSKTNRTLYLFG		73	Homolog CD66c-12
CD66f(11)-13	YLFQVTKYIAGPYE		191	
CD66f(11)-14	IRNPVSASRSDPVT		192	

CD66f(11)-15	SDPVTLNLLPKLPI		74	Homolog CD66a-15
CD66f(11)-16	INNLPRENKDVLA		75	Homolog CD66a-16
CD66f(11)-17	EPKSENYTYIWWLN		76	Homolog CD66a-17
CD66f(11)-18	WLNQSLPVSPGVK		77	Homolog CD66a-18
CD66f(11)-19	RPIENRILLPVS		78	Homolog CD66a-19, CD66c-19
CD66f(11)-20	NETGPYQCEIRDRYG		193	
CD66f(11)-21	DRYGGLRSNPVILN		79	Homolog CD66a-21
CD66f(11)-22	RSNPVILNVLYGPD		194	
CD66f(11)-23	DLPRIYPSFTYYRS		80	Homolog CD66a-23
CD66f(11)-24	TESPPAEYFWTIN		81	Homolog CD66a-24
CD66f(11)-25	INGKFQSQGQKLFI		195	
CD66f(11)-26	KLFPQITRNHSGI		82	Homolog CD66a-26
CD66f(11)-27	SVHNSATGKEISKS		196	
CD66f(11)-28	KEISKSMTVKVSIGK		197	
CD66f(11)-29	KWIPASLAVGFYVE		83	Homolog CD66e-31

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CD66(11)-5L	DGKIIYGPAYSGR	Shift CD66(11)-5 to left	198	
CD66(11)-6L	TVYSNASLLIQNVT	Shift CD66(11)-6 to left	84	Homolog CD66a-6L

Table IX: Peptides Derived from Homology Loops of CD66 Family Members

CD66a Peptide Name	CD66a SEQ ID NO:	CD66a Peptide Name	CD66a SEQ ID NO:	CD66b Peptide Name	CD66b SEQ ID NO:	CD66c Peptide Name	CD66c SEQ ID NO:	CD66d Peptide Name	CD66d SEQ ID NO:	CD66e Peptide Name	CD66e SEQ ID NO:	CD66f(1) Peptide Name	CD66f(1) SEQ ID NO:	CD66f(11) Peptide Name	CD66f(11) SEQ ID NO:
CD66a -1	1	CD66b -1	18	CD66c -1	29	CD66d -1	29	CD66e -1	29	CD66f(1) -1	56	CD66f(11) -1	68		
CD66a -2	2	CD66b -2	19	CD66c -2	30	CD66d -2	39	CD66e -2	39	CD66f(1) -2	57	CD66f(11) -2	69		
CD66a -3	3	CD66b -3	20	CD66c -3	31	CD66d -3	31	CD66e -3	41	CD66f(1) -3	58	CD66f(11) -3	70		
CD66a -4	117	CD66b -4	141	CD66c -4	150	CD66d -4	150	CD66e -4	159	CD66f(1) -4	176	CD66f(11) -4	185		
CD66a -5	118	CD66b -5	142	CD66c -5	151	CD66d -5	156	CD66e -5	160	CD66f(1) -5	177	CD66f(11) -5	186		
CD66a -6	119	CD66b -6	143	CD66c -6	119	CD66d -6	157	CD66e -6	161	CD66f(1) -6	178	CD66f(11) -6	187		
CD66a -7	4	CD66b -7	21	CD66c -7	4	CD66d -7	4	CD66e -7	4	CD66f(1) -7	59	CD66f(11) -7	71		
CD66a -8	120	CD66b -8	144	CD66c -8	120	CD66d -8	158	CD66e -8	162	CD66f(1) -8	179	CD66f(11) -8	188		
								CD66e -9	163						
CD66a -9	121	CD66b -9	121	CD66c -9	121			CD66e -10	164	CD66f(1) -9	180	CD66f(11) -9	189		
CD66a -10	5	CD66b -10	22	CD66c -10	32			CD66e -11	42	CD66f(1) -10	60	CD66f(11) -10	72		
CD66a -11	122	CD66b -11	145	CD66c -11	145			CD66e -12	122	CD66f(1) -11	181	CD66f(11) -11	190		
CD66a -12	6	CD66b -12	6	CD66c -12	33			CD66e -13	43	CD66f(1) -12	61	CD66f(11) -12	73		

CD66a -13	123	CD66b -13	146	CD66c -13	152				CD66e -14	165	CD66f(1) -13	182	CD66f(11) -13	191
CD66a -14	124	CD66b -14	147	CD66c -14	153				CD66e -15	166	CD66f(1) -14	183	CD66f(11) -14	192
CD66a -15	7	CD66b -15	7	CD66c -15	7				CD66e -16	44	CD66f(1) -15	62	CD66f(11) -15	74
									CD66e -17	45				
CD66a -16	8	CD66b -16	23	CD66c -16	34				CD66e -18	46	CD66f(1) -16	63	CD66f(11) -16	75
CD66a -17	9	CD66b -17	24	CD66c -17	35				CD66e -19	47	CD66f(1) -17	64	CD66f(11) -17	76
CD66a -18	10	CD66b -18	25	CD66c -18	36				CD66e -20	48	CD66f(1) -18	65	CD66f(11) -18	77
CD66a -19	11	CD66b -19	26	CD66c -19	37				CD66e -21	11	CD66f(1) -19	66	CD66f(11) -19	78
CD66a -20	125	CD66b -20	148	CD66c -20	154				CD66e -22	167	CD66f(1) -20	184	CD66f(11) -20	193
CD66a -21	12	CD66b -21	27	CD66c -21	38				CD66e -23	49	CD66f(1) -21	67	CD66f(11) -21	79
CD66a -22	126								CD66e -24	168			CD66f(11) -22	194
CD66a -23	13								CD66e -25	50			CD66f(11) -23	80
CD66a -24	14								CD66e -26	51			CD66f(11) -24	81
CD66a -25	127								CD66e -27	122			CD66f(11) -25	195
CD66a -26	15								CD66e -28	52			CD66f(11) -26	82
CD66a -27	128								CD66e -29	146			CD66f(11) -27	196

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Sequence Free Text

25 SEQ ID NOs:1-200 Synthetic Peptides

The complete disclosure of all patents, patent documents, and publications cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

WHAT IS CLAIMED IS:

1. An isolated peptide comprising an amino acid sequence represented by
SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof
5 that modulate the function of at least one CD66 family member and/or at
least one ligand thereof.
2. The peptide of claim 1 represented by SEQ ID NOs:1-100, 119, 143,
157, 161, 178, or 187.
- 10 3. The peptide of claim 2 represented by SEQ ID NOs:1, 2, 3, 4, 7-15, 17,
22, 32, 33, 35, 37, 39, 41, 47, 53, or 54.
4. The peptide of claim 1 which is capable of modulating at least one of the
15 following:
 - activation of neutrophils;
 - activation or inhibition of T-cells, B-cells, NK cells, LAK cells,
dendritic cells, or other immune system cells;
 - proliferation and/or differentiation of T-cells, B-cells, NK cells,
20 LAK cells, dendritic cells, or other immune system cells;
 - proliferation and/or differentiation of epithelial cells;
 - homotypic and/or heterotypic adhesion among CD66 family
members; and
 - adhesion of CD66 family members to other ligands.
- 25 5. The peptide of claim 1 which is complexed with a carrier molecule or
structure to form a peptide conjugate.
6. The peptide of claim 5 wherein the carrier molecule or structure is
30 selected from the group of microbeads, liposomes, biological carrier
molecules, synthetic polymers, biomaterials, and cells.

7. The peptide of claim 6 wherein the peptide conjugate binds to cells expressing a CD66 protein or a CD66 ligand.
8. The peptide of claim 5 wherein the peptide conjugate includes a label.
9. The peptide of claim 1 which is attached to a label.
10. The peptide of claim 9 wherein the label is selected from the group consisting of a fluorescent tag, a radioactive tag, a magnetic resonance tag, an enzymatic tag, and combinations thereof.
11. A method of activating a neutrophil comprising contacting the neutrophil with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1, 2, 3, 4, 17, 41, or analogs thereof.
12. The method of claim 11 wherein the peptide is represented by SEQ ID NOs:1, 2, 3, 4, 17, or 41.
13. The method of claim 11 which is carried out *in vitro*.
14. The method of claim 11 which is carried out *in vivo*.
15. A method of blocking the activation of a neutrophil induced by the method of claim 11, the method comprising contacting the neutrophil when in the presence of at least one of the peptides listed in claim 11 with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, 84, or analogs thereof.
16. The method of claim 15 wherein the peptide is represented by SEQ ID NOs:18-21, 28-31, 39, 40, 55-59, 68-71, or 84.

17. The method of claim 15 which is carried out *in vitro*.
18. The method of claim 15 which is carried out *in vivo*.
- 5 19. A method of modulating the homotypic and/or heterotypic adhesion of CD66 family members or adhesion of a CD66 protein to a CD66 ligand; the method comprising contacting CD66 family members and/or their ligands with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:7-15, 17, 22, 32, 33,
10 35, 37, 39, 47, 53, 54, or analogs thereof.
20. The method of claim 19 wherein the peptide is represented by SEQ ID NOs:7-15, 17, 22, 32, 33, 35, 37, 39, 47, 53, or 54.
- 15 21. The method of claim 19 which is carried out *in vitro*.
22. The method of claim 19 which is carried out *in vivo*.
- 20 23. A method of altering the modulation of the homotypic and/or heterotypic adhesion of CD66 family members or adhesion between a CD66 protein and a CD66 ligand induced by the method of claim 19, the method comprising contacting CD66 family members and/or their ligands when in the presence of at least one of the peptides listed in claim 19 with at least one peptide or peptide conjugate comprising an
25 amino acid sequence represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, 72-100, or analogs thereof.
- 30 24. The method of claim 23 wherein the peptide is represented by SEQ ID NOs:2, 5, 6, 9, 11, 16, 19, 23-28, 30, 32, 34-38, 40, 42, 43-47, 49-52, 55, 57, 60-67, 69, or 72-100.
25. The method of claim 23 which is carried out *in vitro*.

26. The method of claim 23 which is carried out *in vivo*.
27. A method of modulating immune cell activation, proliferation, and/or
5 differentiation; the method comprising contacting an immune cell with
at least one peptide or peptide conjugate comprising an amino acid
sequence represented by SEQ ID NOs:14, 53, or analogs thereof.
28. The method of claim 27 wherein the peptide is represented by SEQ ID
10 NOs:14 or 53.
29. The method of claim 27 wherein the immune cell is selected from the
group of a T-cell, a B-cell, a LAK cell, an NK cell, a dendritic cell, and
combinations thereof.
- 15 30. The method of claim 27 which is carried out *in vitro*.
31. The method of claim 27 which is carried out *in vivo*.
- 20 32. A method of modulating at least one of the following functions of CD66
family members and/or ligands thereof in cells: activation of
neutrophils; activation or inhibition of T-cells, B-cells, NK cells, LAK
cells, dendritic cells, or other immune system cells; proliferation and/or
differentiation of T-cells, B-cells, LAK cells, NK cells, dendritic cells,
25 or other immune system cells; proliferation and/or differentiation of
epithelial cells; homotypic and/or heterotypic adhesion among CD66
family members; and adhesion of CD66 family members to other
ligands; the method comprising contacting cells with at least one peptide
or peptide conjugate comprising an amino acid sequence represented by
30 SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
33. A method of delivering a therapeutically active agent to a patient
comprising administering at least one peptide conjugate comprising a

peptide and the therapeutically active agent to a patient wherein the peptide comprises an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

- 5 34. The method of claim 33 wherein the therapeutically active agent is selected from drugs, DNA sequences, RNA sequences, proteins, lipids, and combinations thereof.
- 10 35. The method of claim 33 wherein the therapeutically active agent is an antibacterial agent, antiinflammatory agent, or antineoplastic agent.
- 15 36. A method of modifying the metastasis of malignant cells comprising contacting the malignant cells or normal host tissue with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 20 37. A method of altering bacterial or viral binding to cells or a biomaterial, the method comprising contacting the cells or biomaterial with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 25 38. A method of altering cell adhesion to a biomaterial, the method comprising contacting the biomaterial with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 30 39. A method of detecting tumors comprising contacting tumor cells or tumor vasculature with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.

40. A method of detecting inflammation comprising contacting inflamed vasculature or leukocytes with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 5
41. A method of detecting a CD66 protein or a ligand thereof, the method comprising contacting tissue comprising a CD66 protein or a ligand thereof with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 10
42. A method of altering angiogenesis comprising contacting endothelial cells, tumor cells, or immune cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 15
43. A method of altering an immune response, the method comprising contacting immune system cells with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 20
44. A method of altering keratinocyte proliferation comprising contacting keratinocytes with at least one peptide or peptide conjugate comprising an amino acid sequence represented by SEQ ID NOs:1-100, 119, 143, 157, 161, 178, 187, or analogs thereof.
- 25
45. An isolated peptide comprising an amino acid sequence represented by SMPFN (SEQ ID NO:101), PQQLF (SEQ ID NO:102), LPQQL (SEQ ID NO:103), QQLFG (SEQ ID NO:104), NRQIV (SEQ ID NO:105), GNRQI (SEQ ID NO:106), IKSDLVNE (SEQ ID NO:107), AASNPP (SEQ ID NO:108), NTTYLWWVNG (SEQ ID NO:109), YLWWVNG (SEQ ID NO:110), SWLIN (SEQ ID NO:111), SWFIN (SEQ ID NO:112), AQYSWLIN (SEQ ID NO:113), AQYSWFIN (SEQ ID NO:114), SWFVN (SEQ ID NO:115), AQYSWFIN (SEQ ID NO:116),
- 30

NRQII (SEQ ID NO:199), GNRQI (SEQ ID NO:200), or analogs thereof.

5

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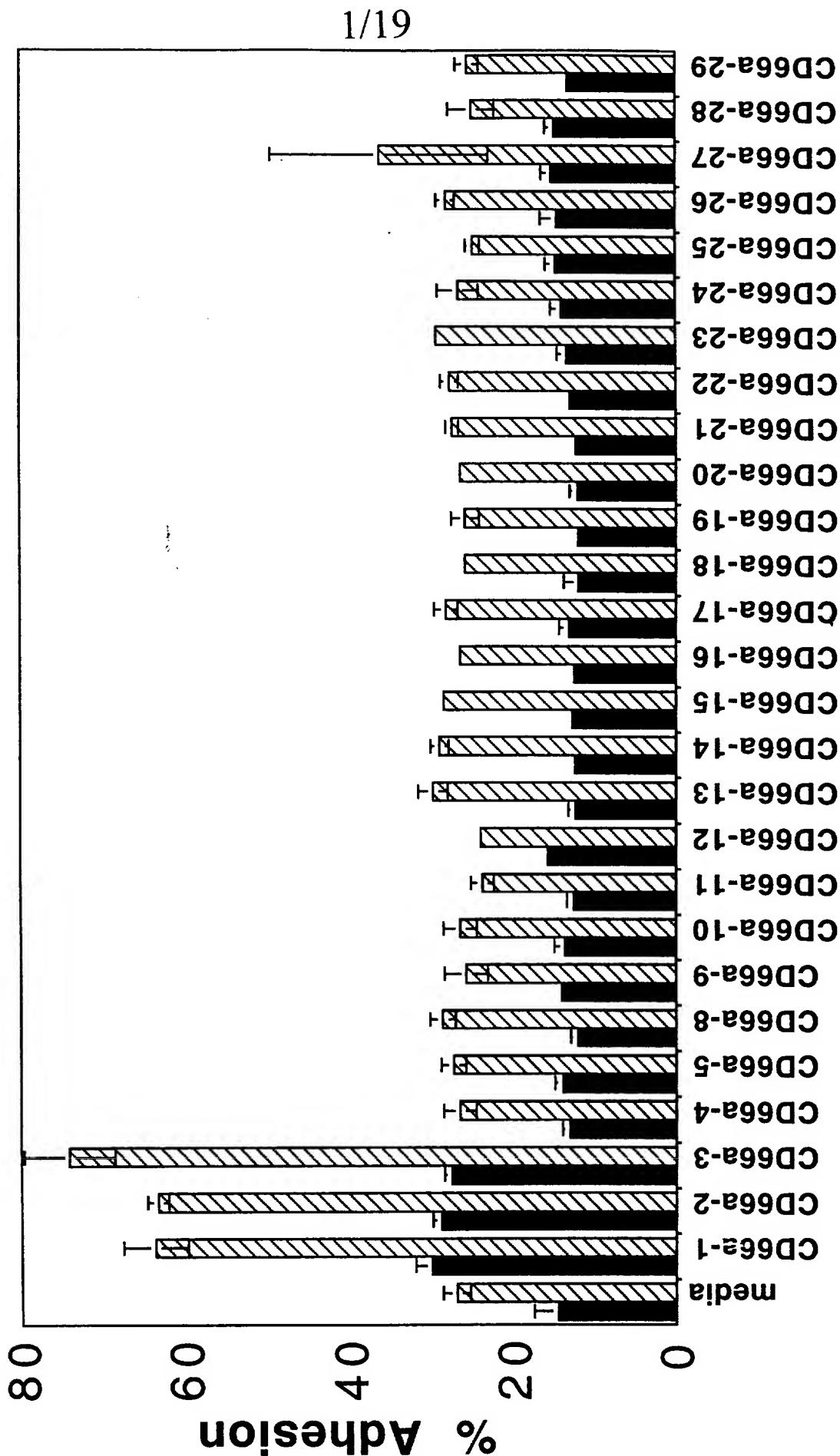
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(54) Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

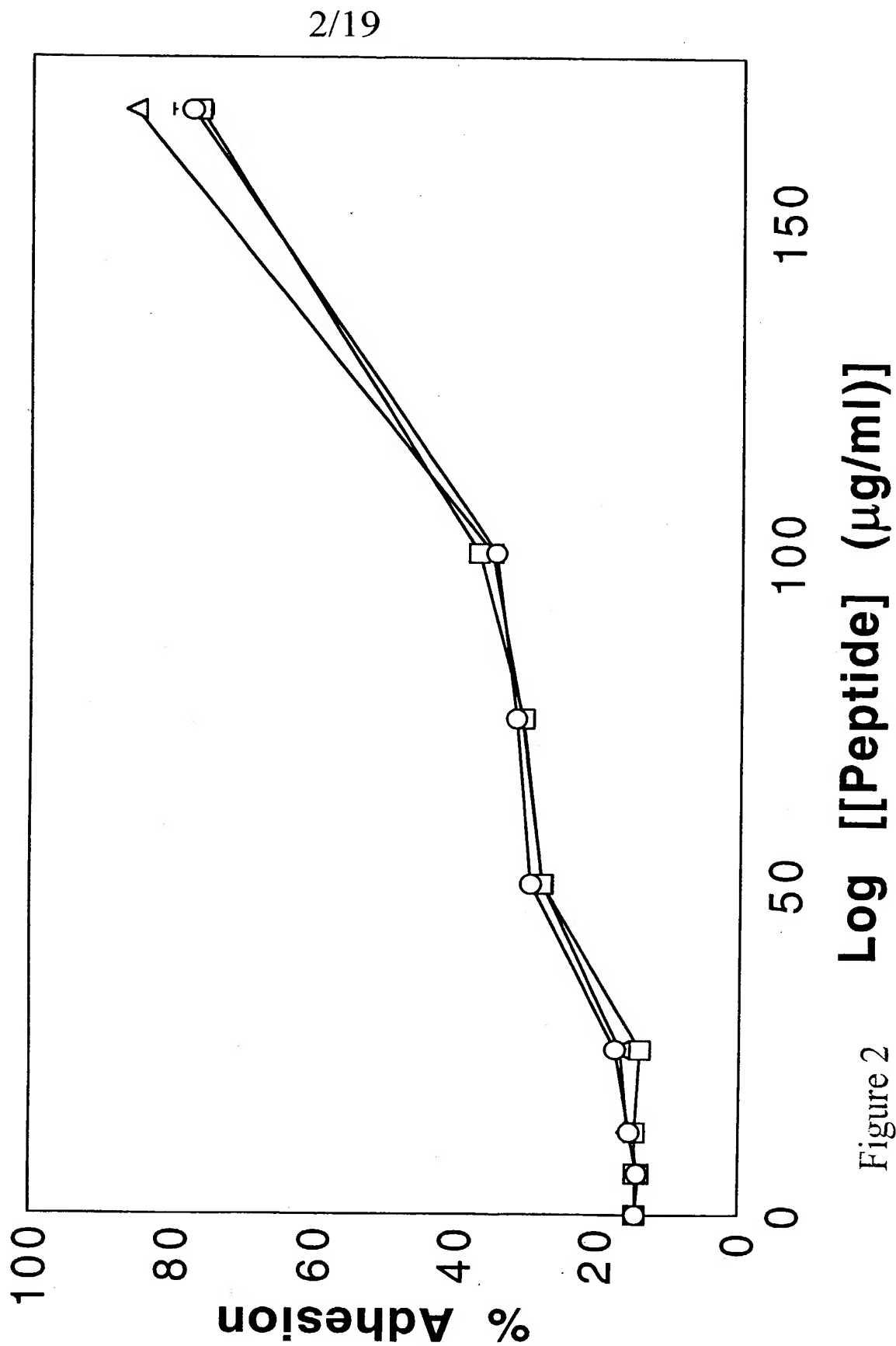
(57) Abstract: The present invention relates to peptides capable of modulating the function (e.g., signaling or adhesive activities) of CD66 (CEACAM) family members and/or their ligands.

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Peptide

Figure 1



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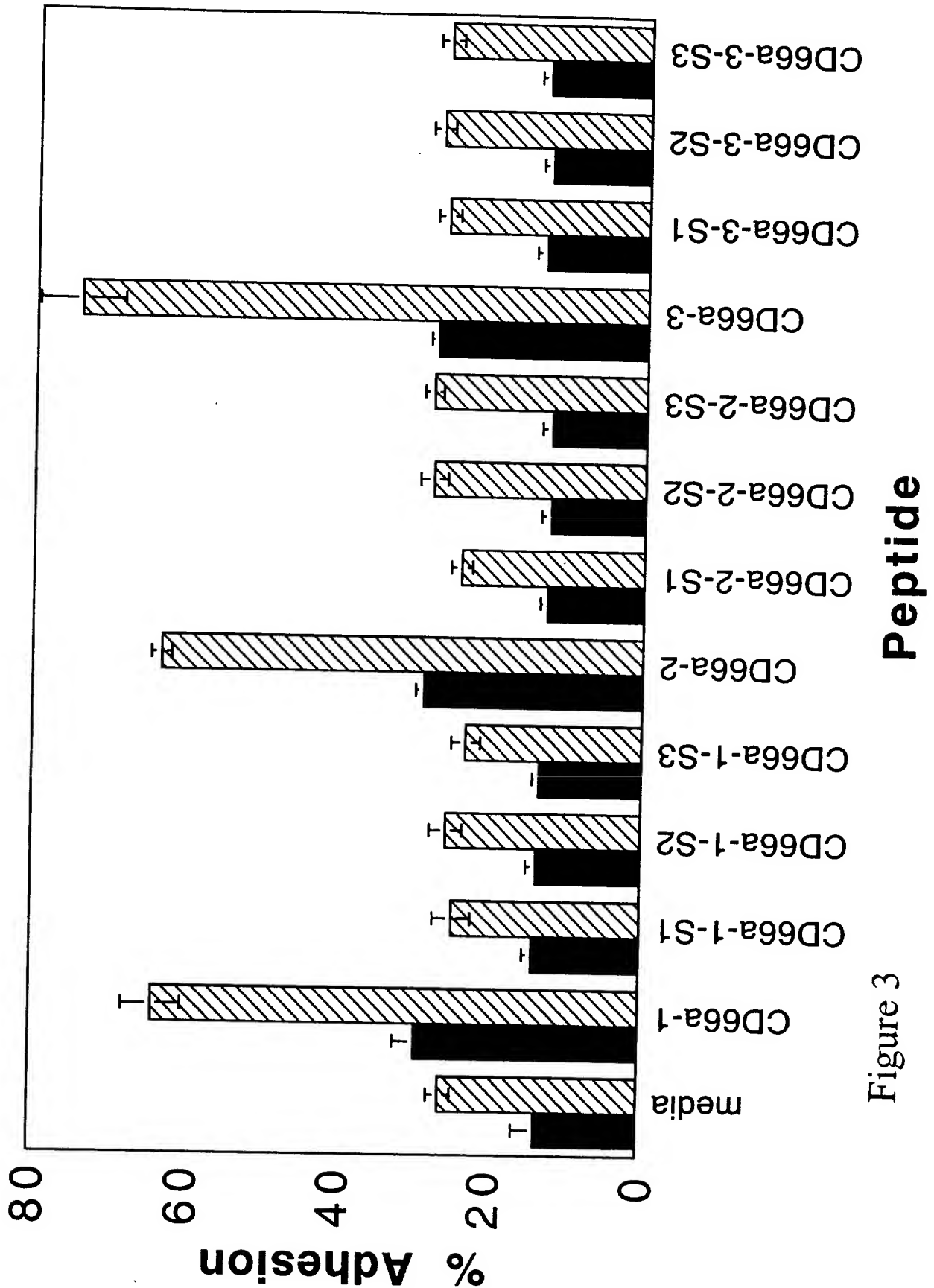


Figure 3

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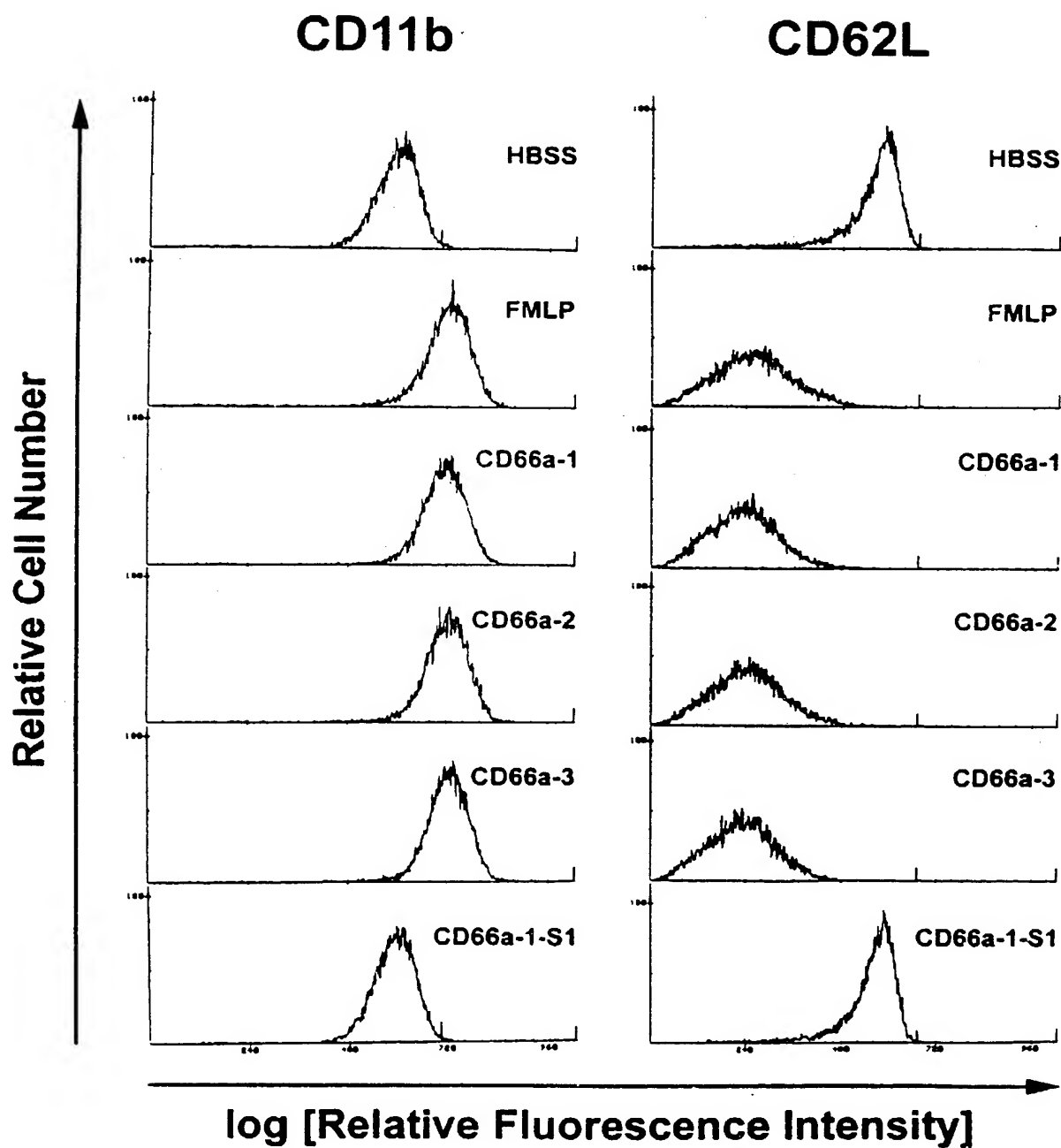


Figure 4

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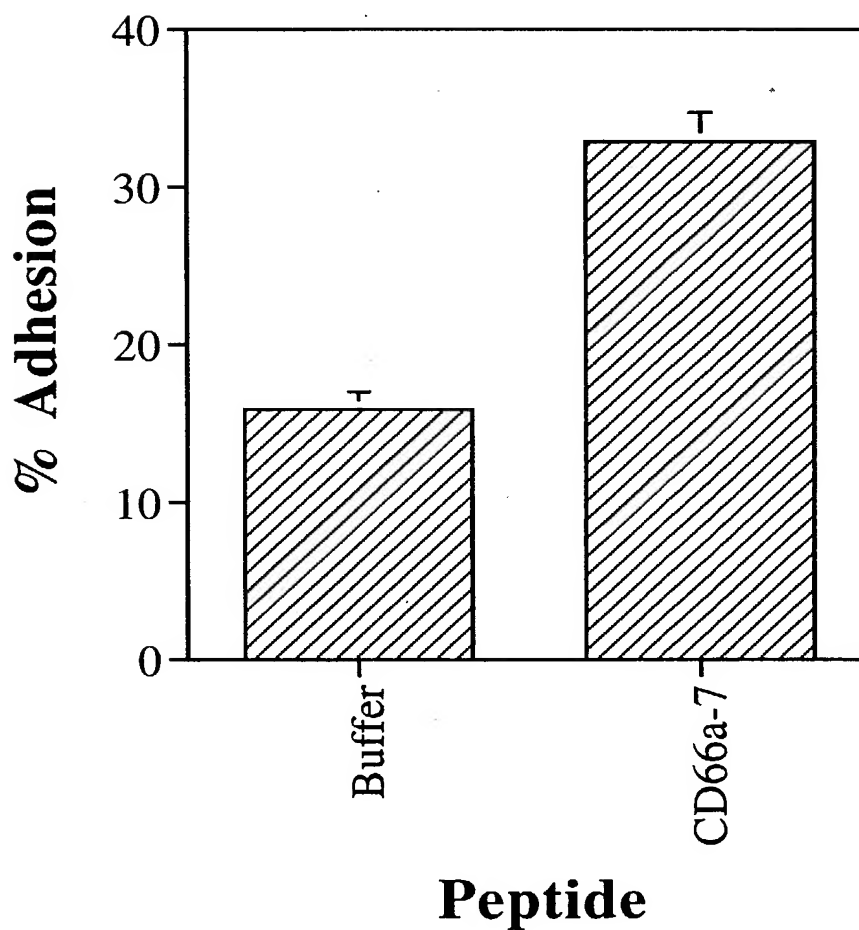


Figure 5

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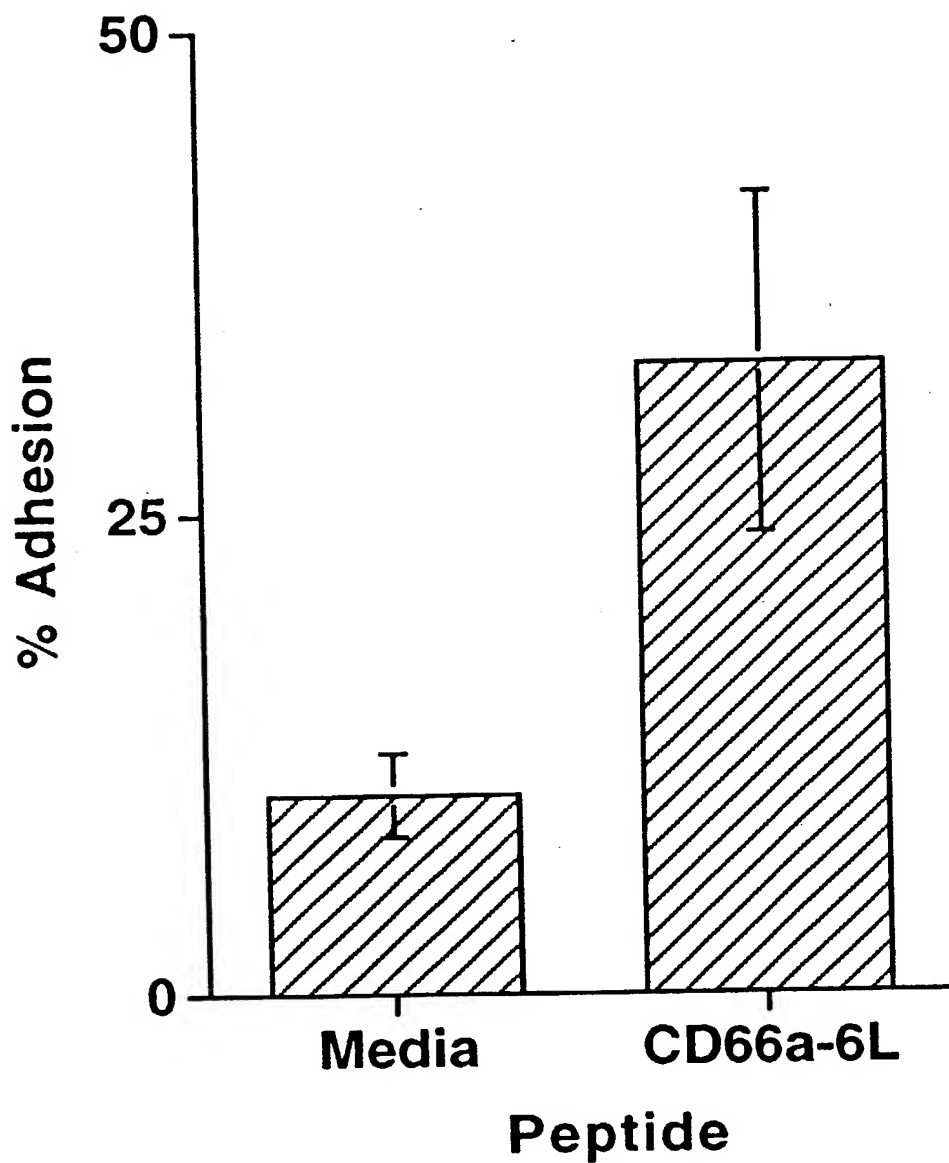


Figure 6

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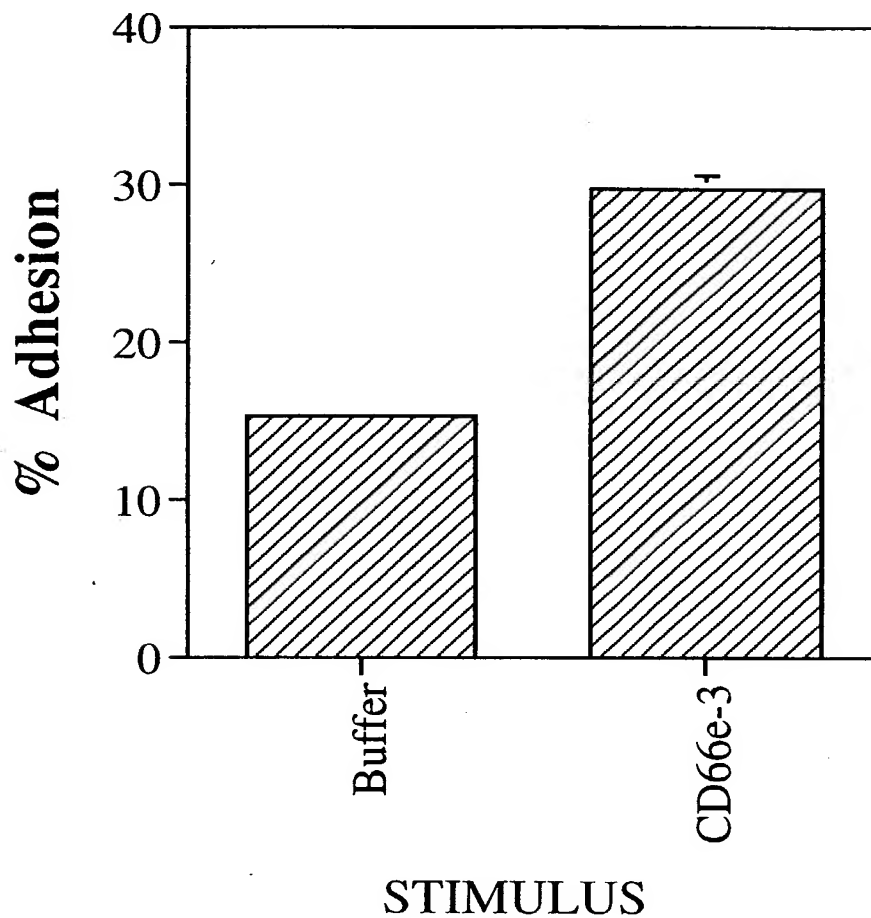


Figure 7

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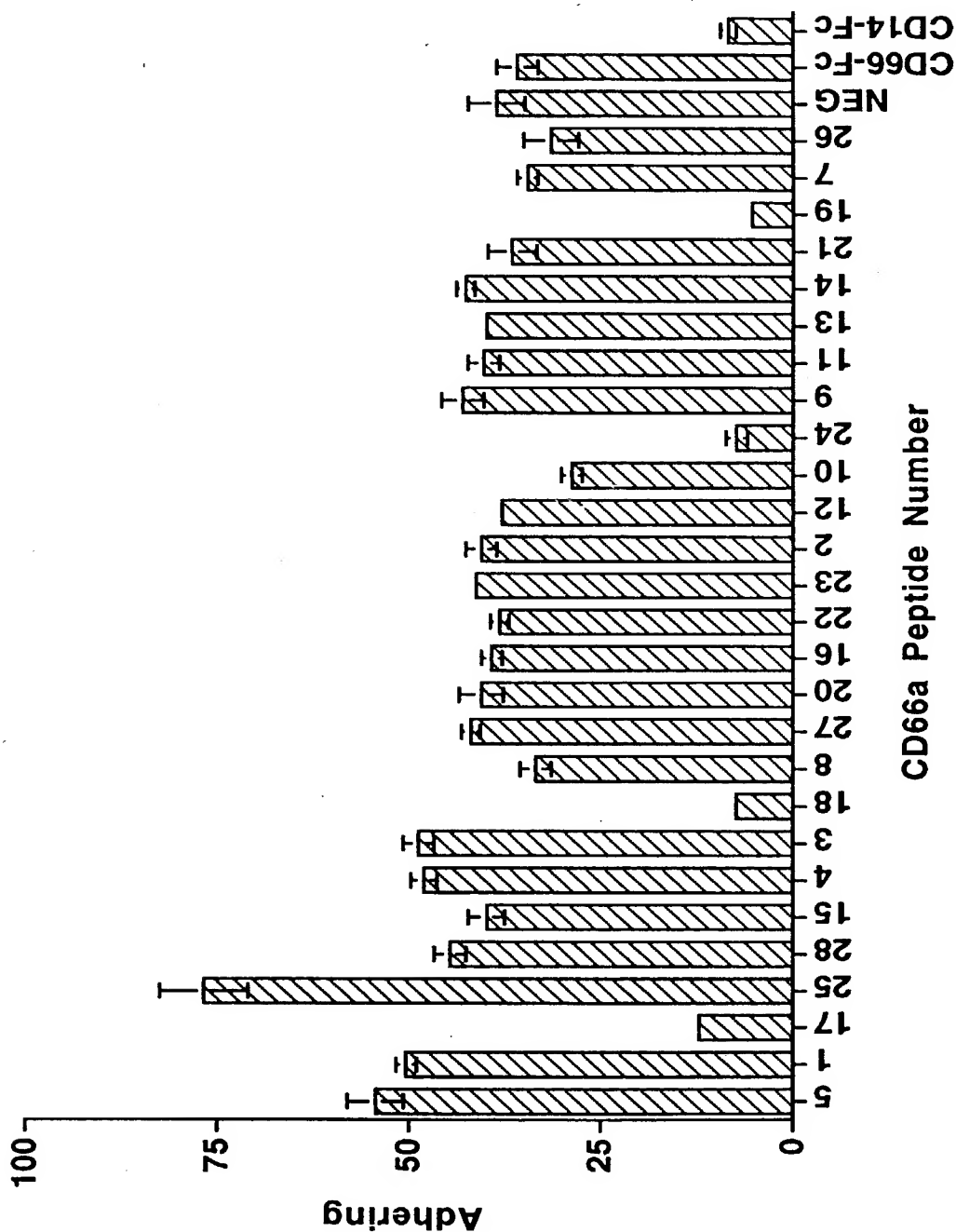


Figure 8

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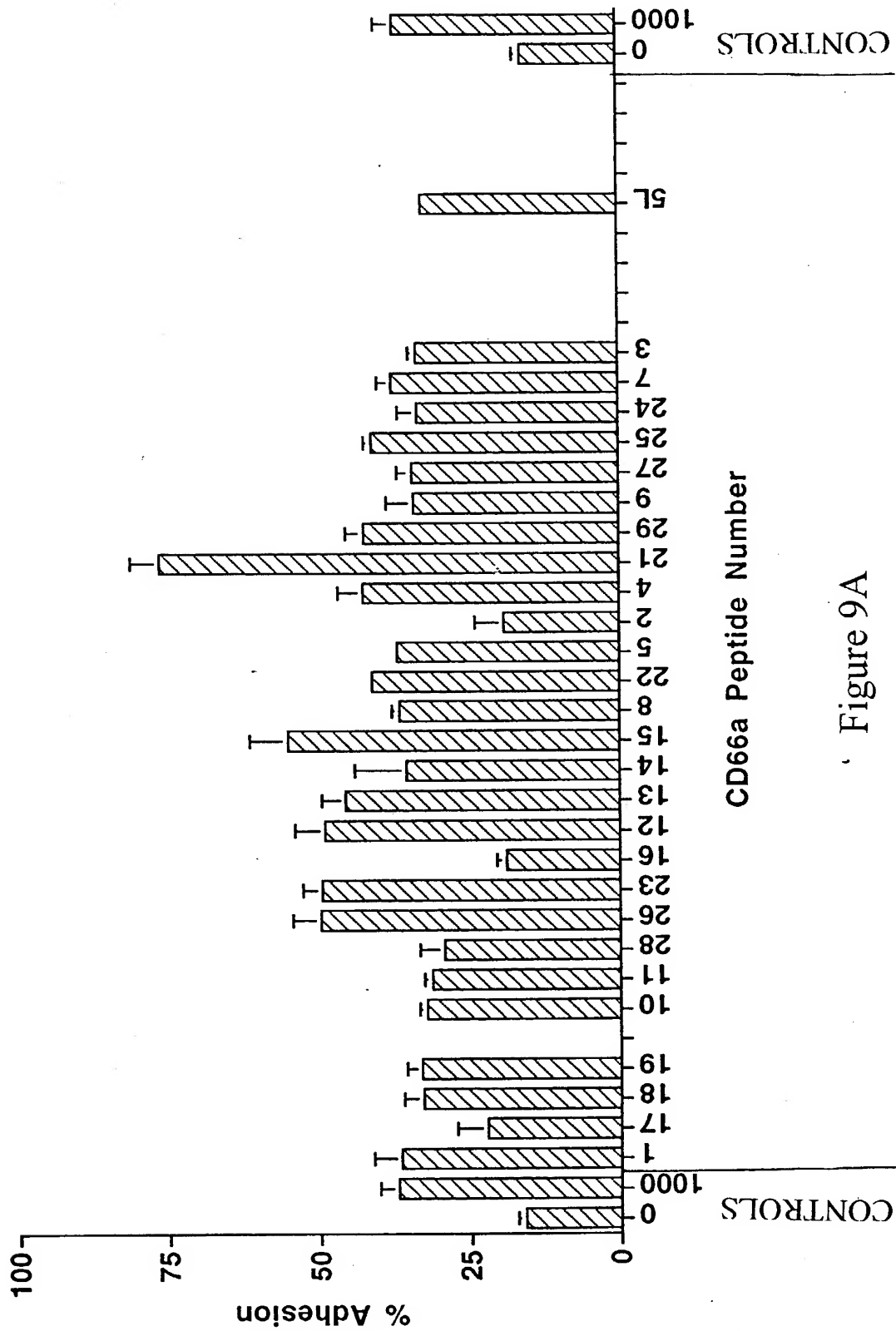


Figure 9A

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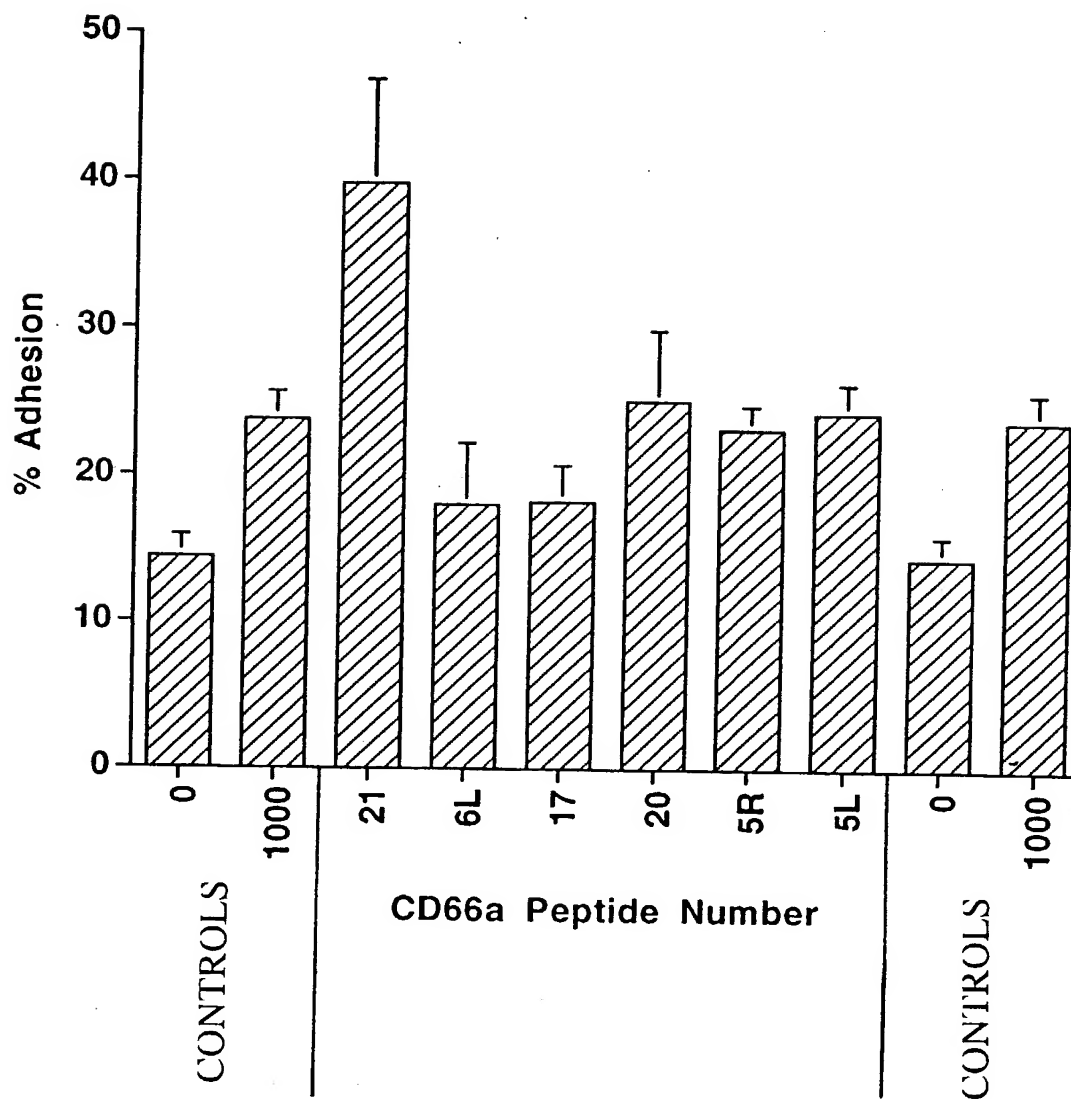
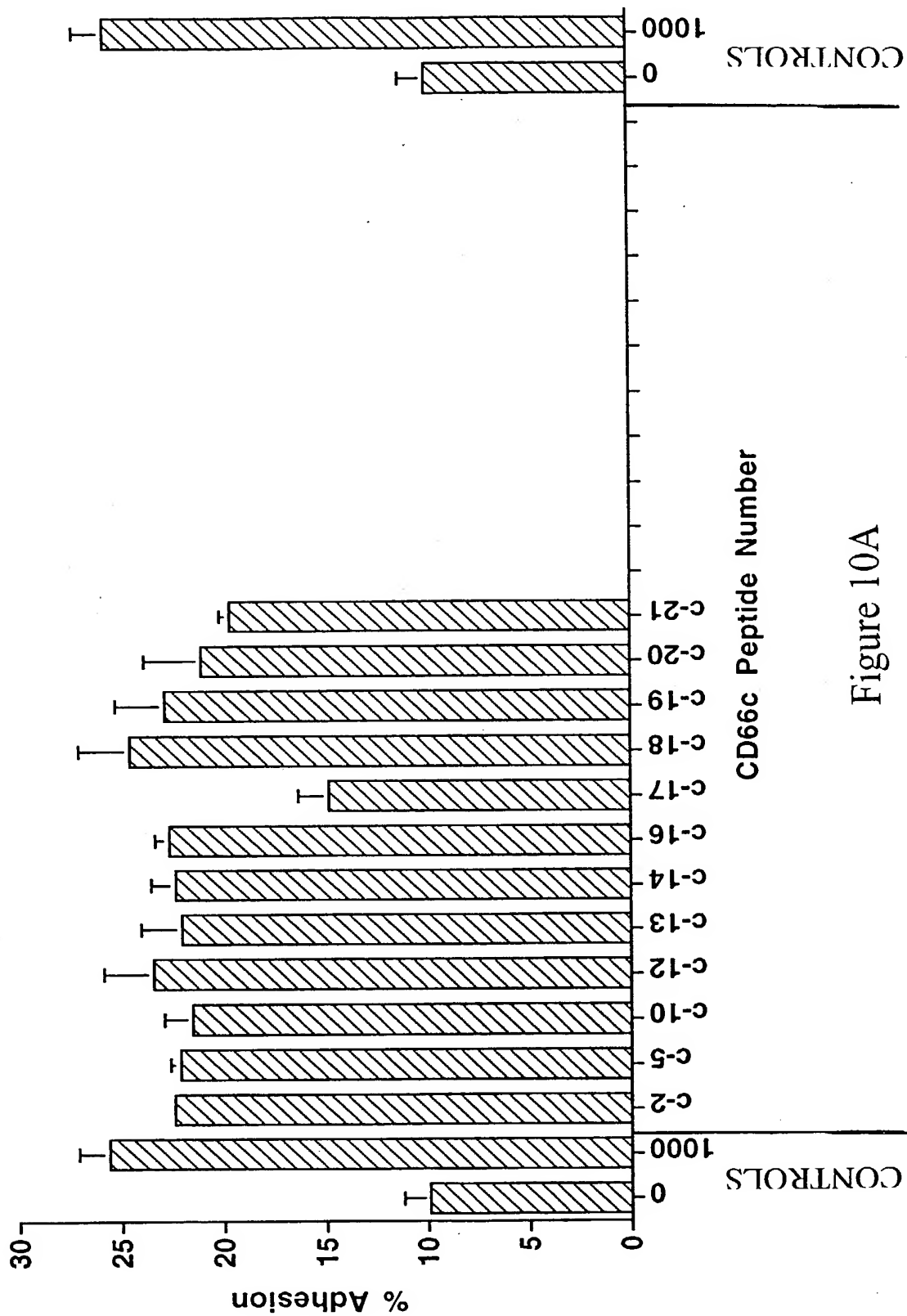


Figure 9B

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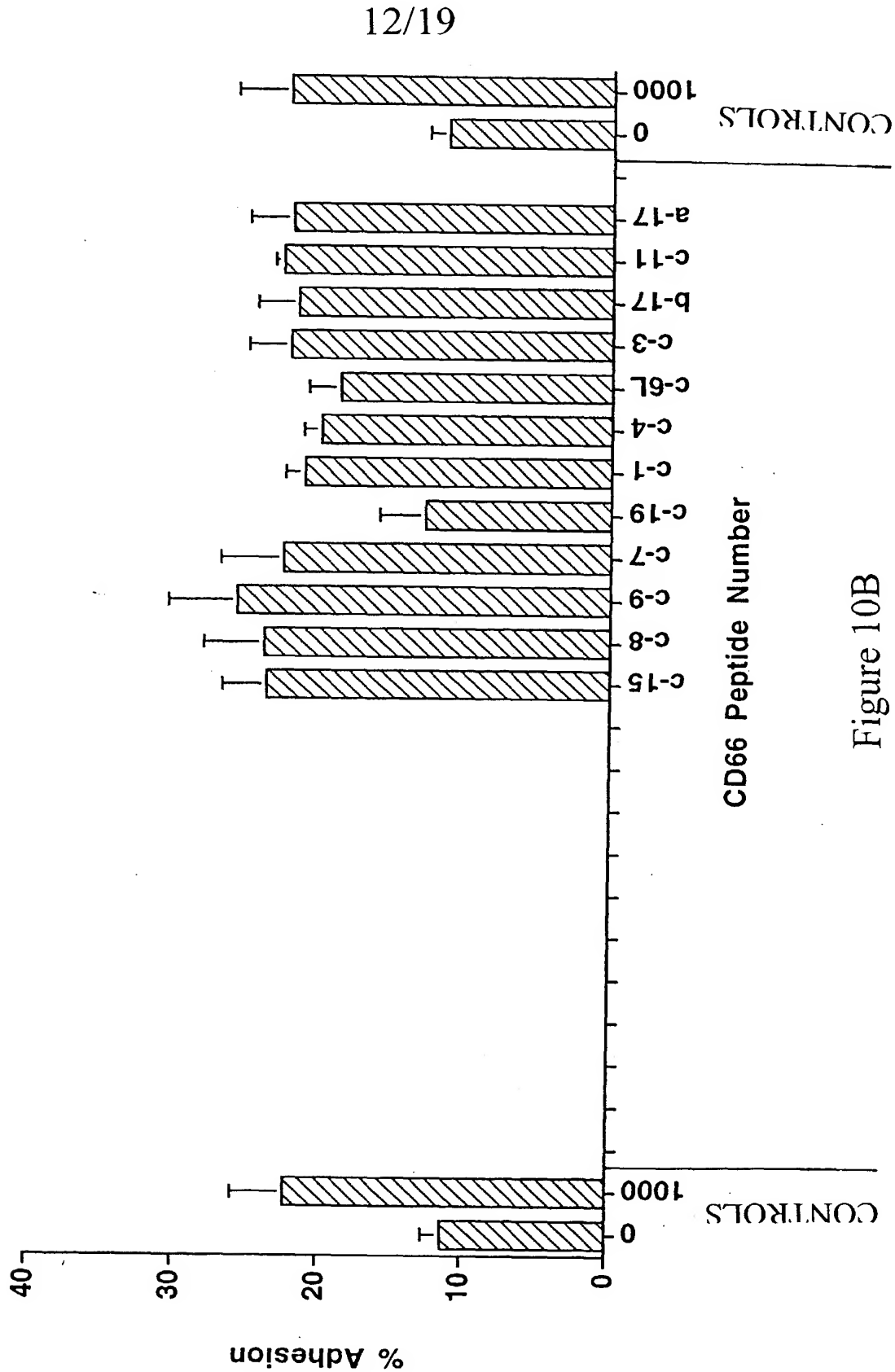


Figure 10B

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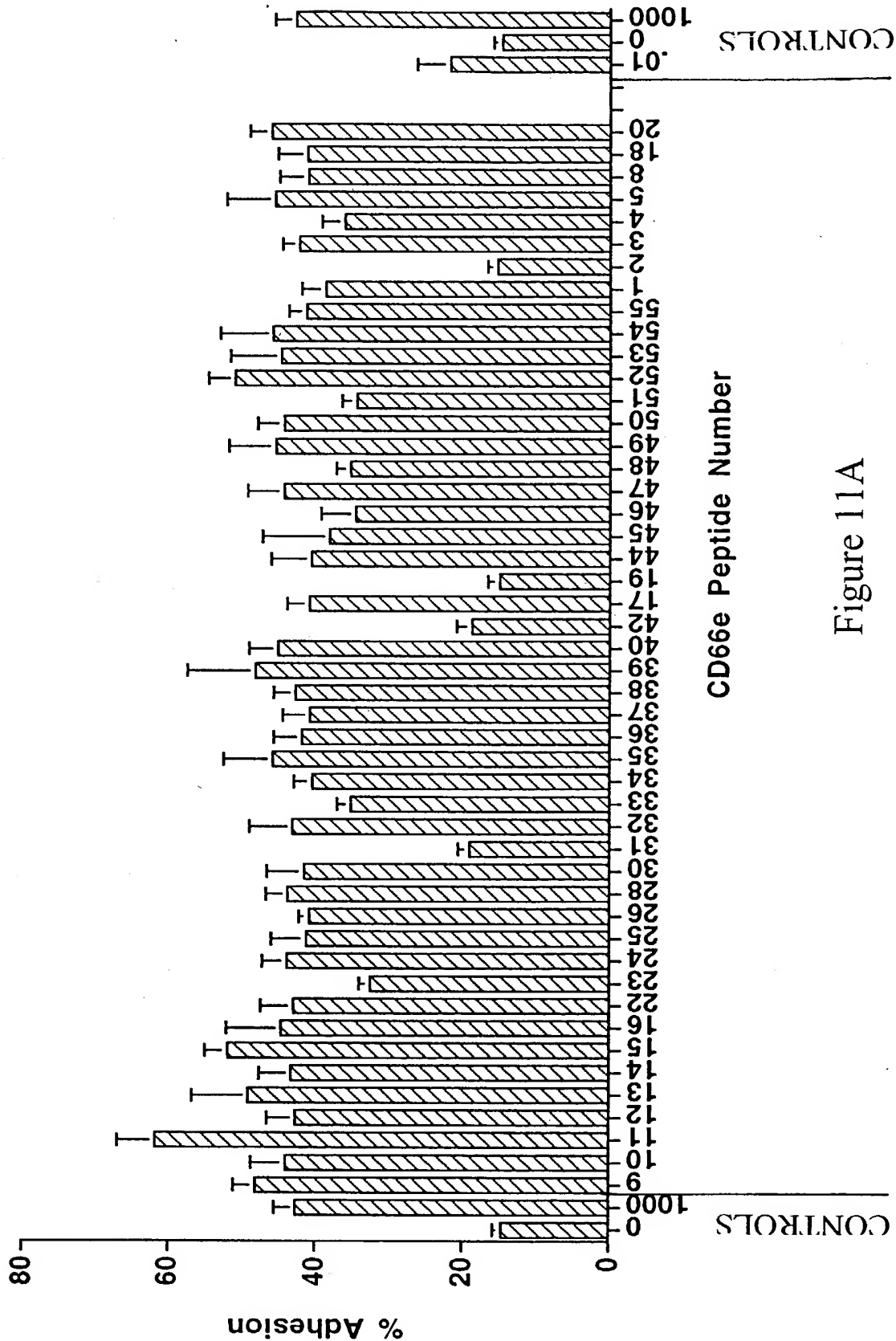
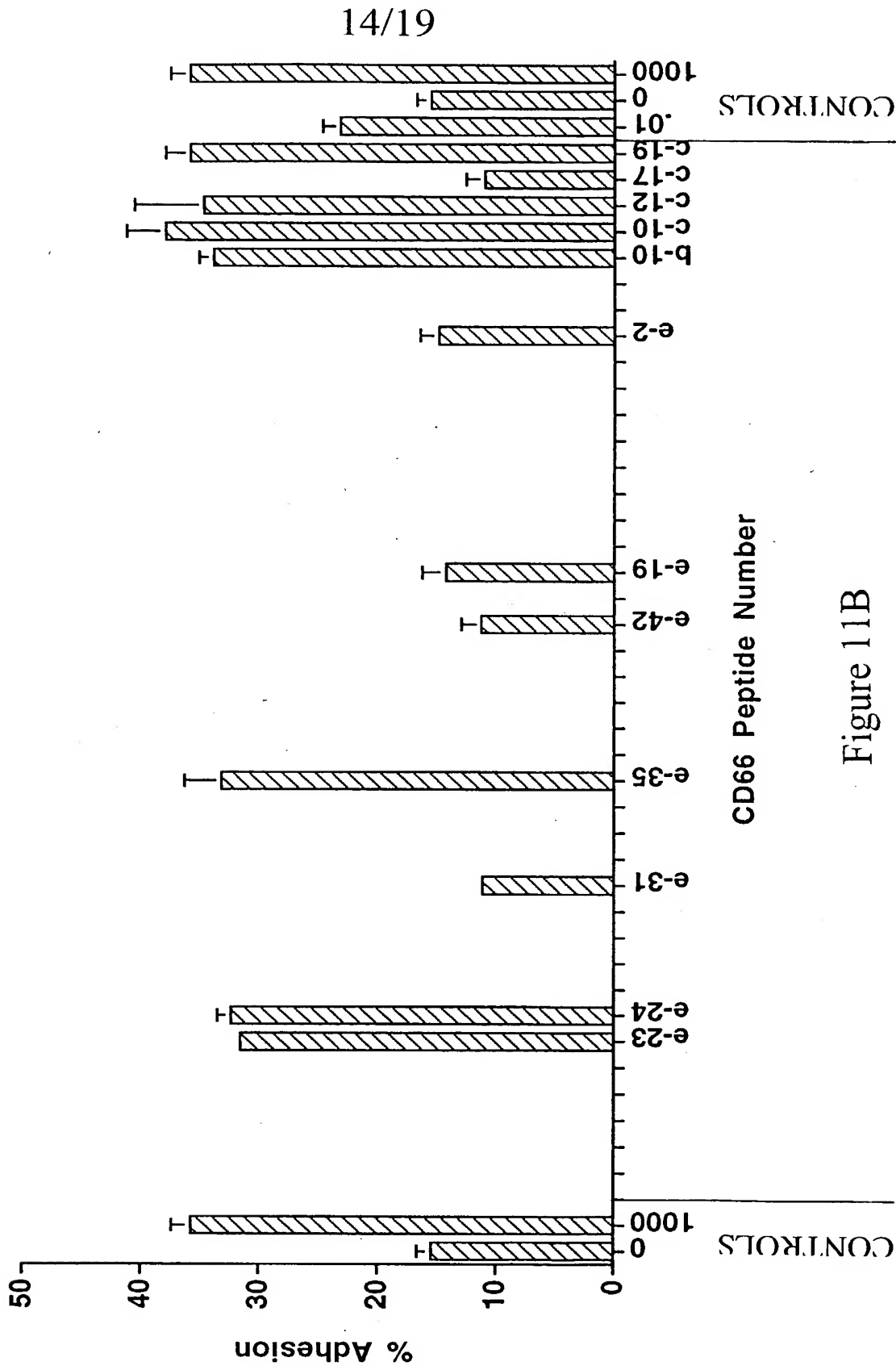


Figure 11A

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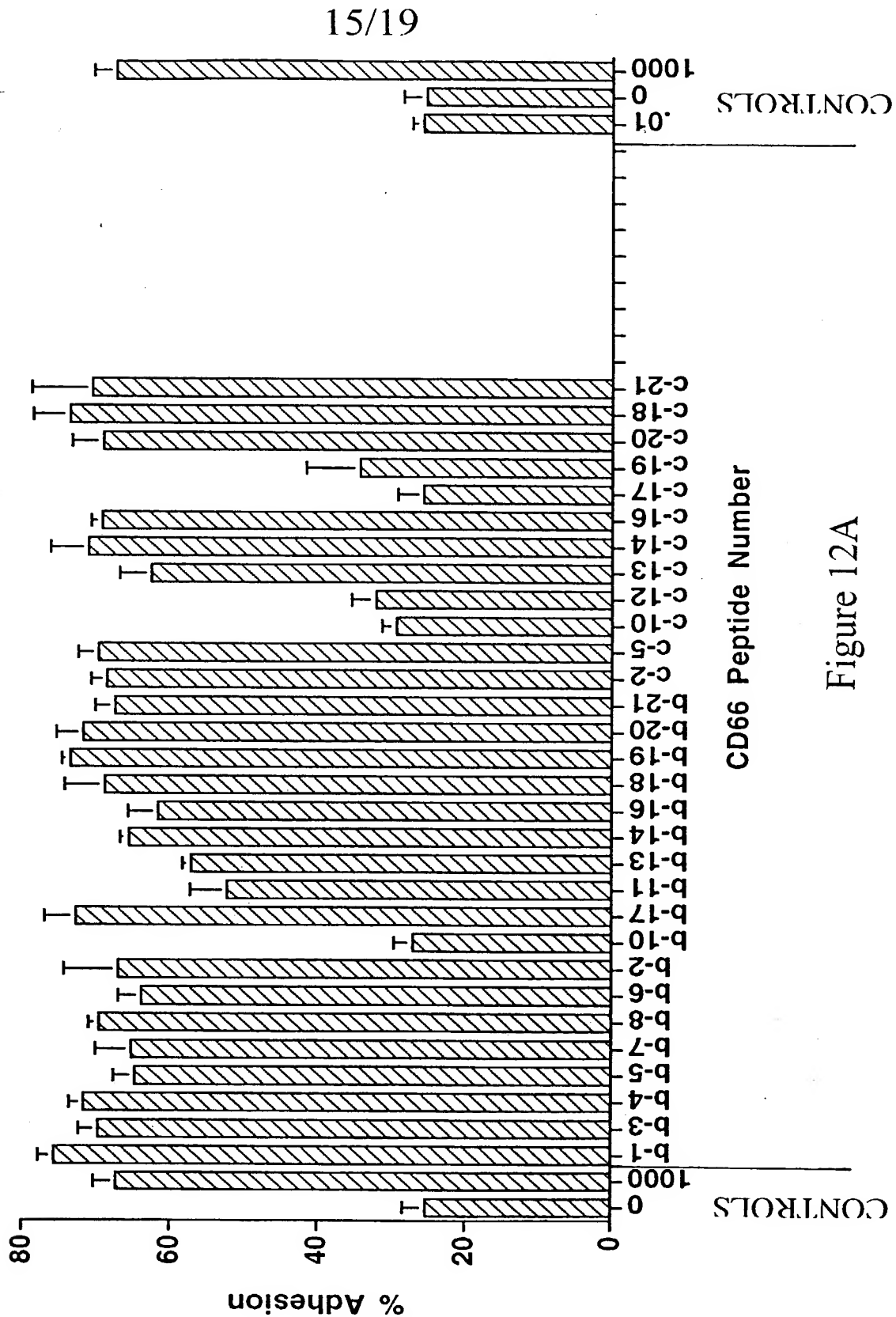


Figure 12A

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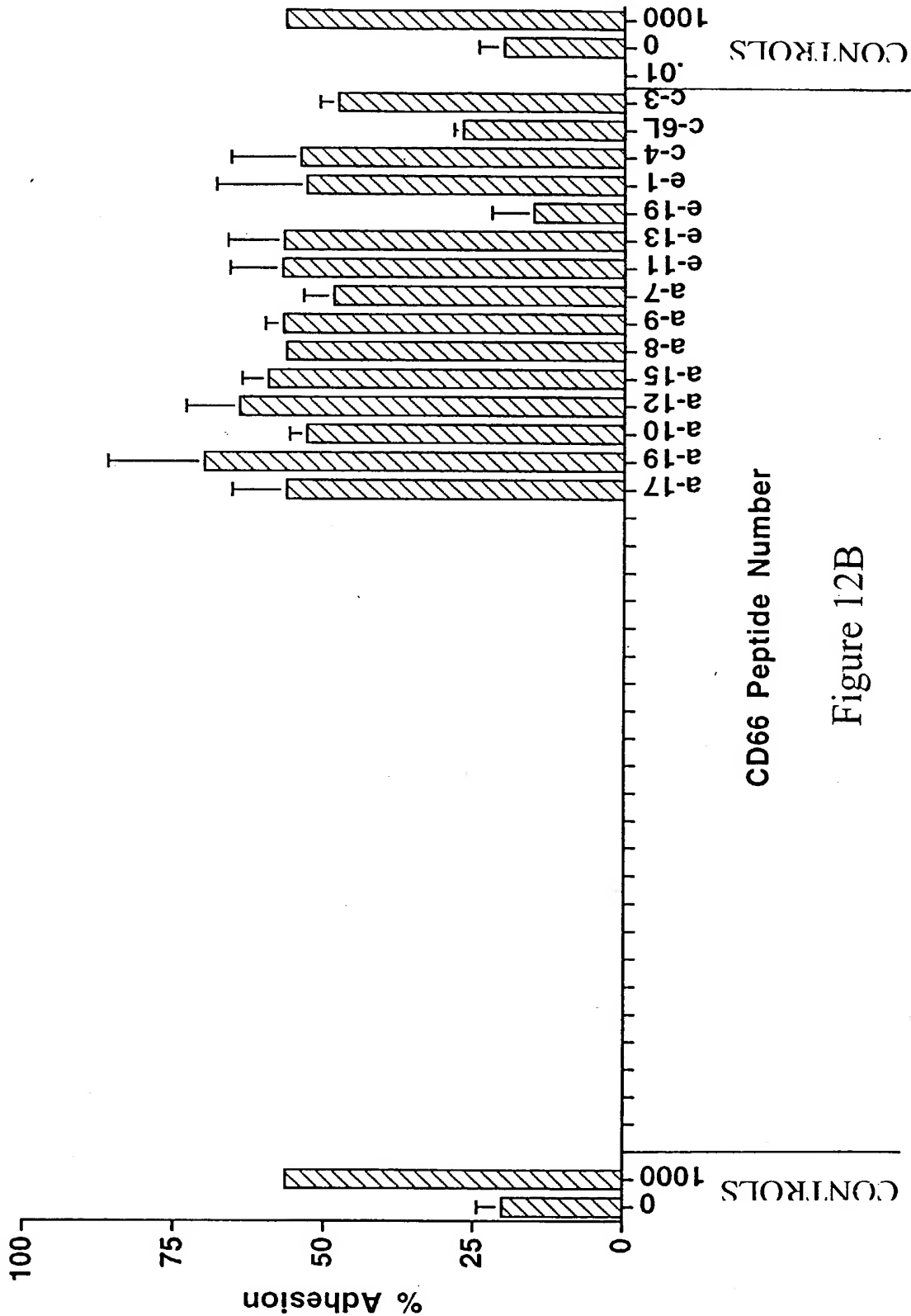


Figure 12B

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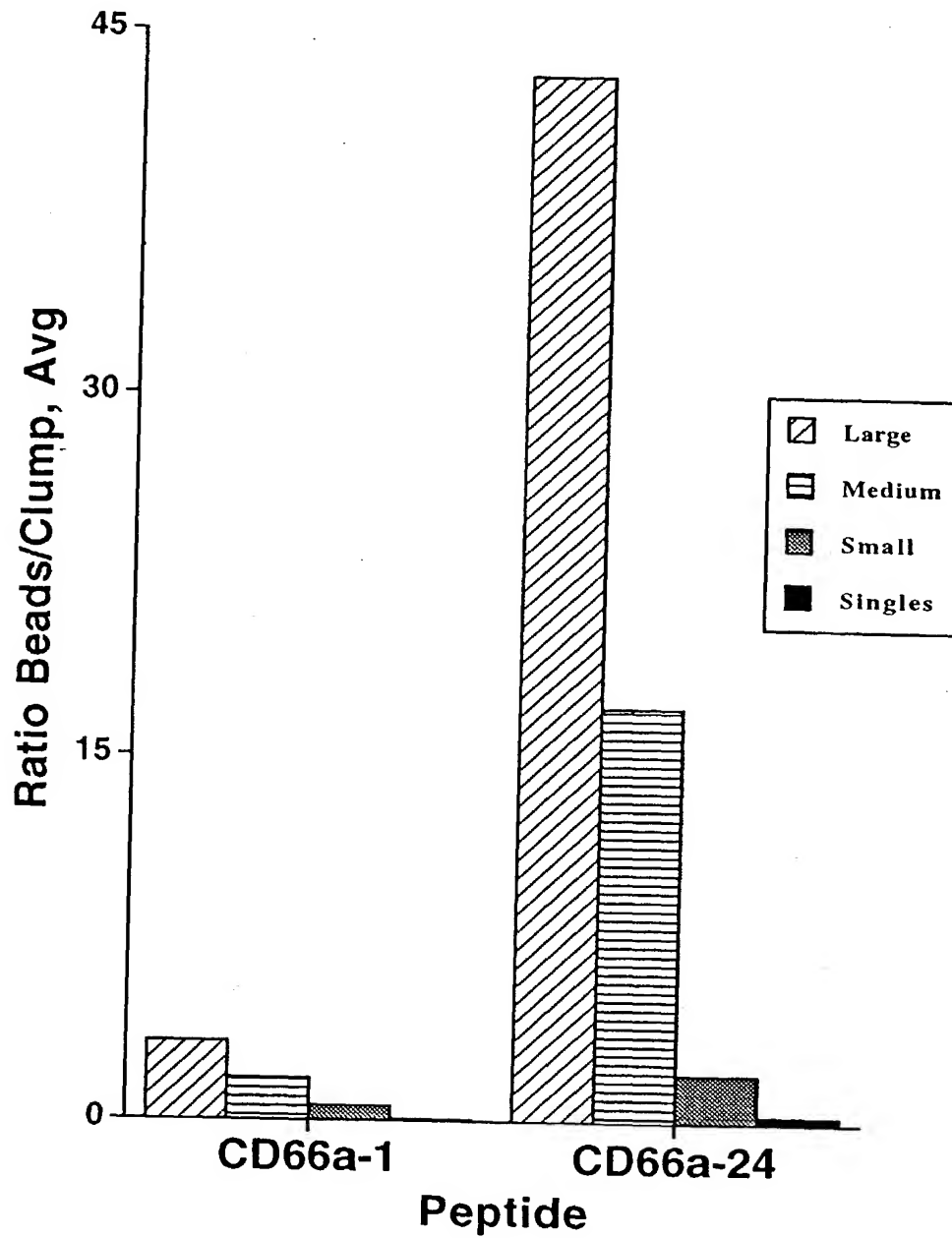


Figure 13A

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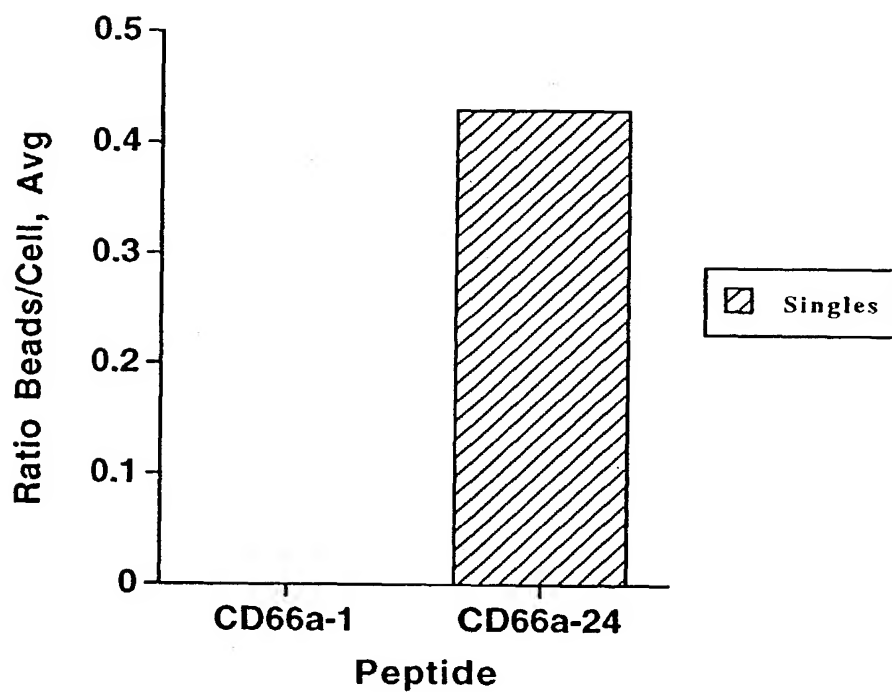
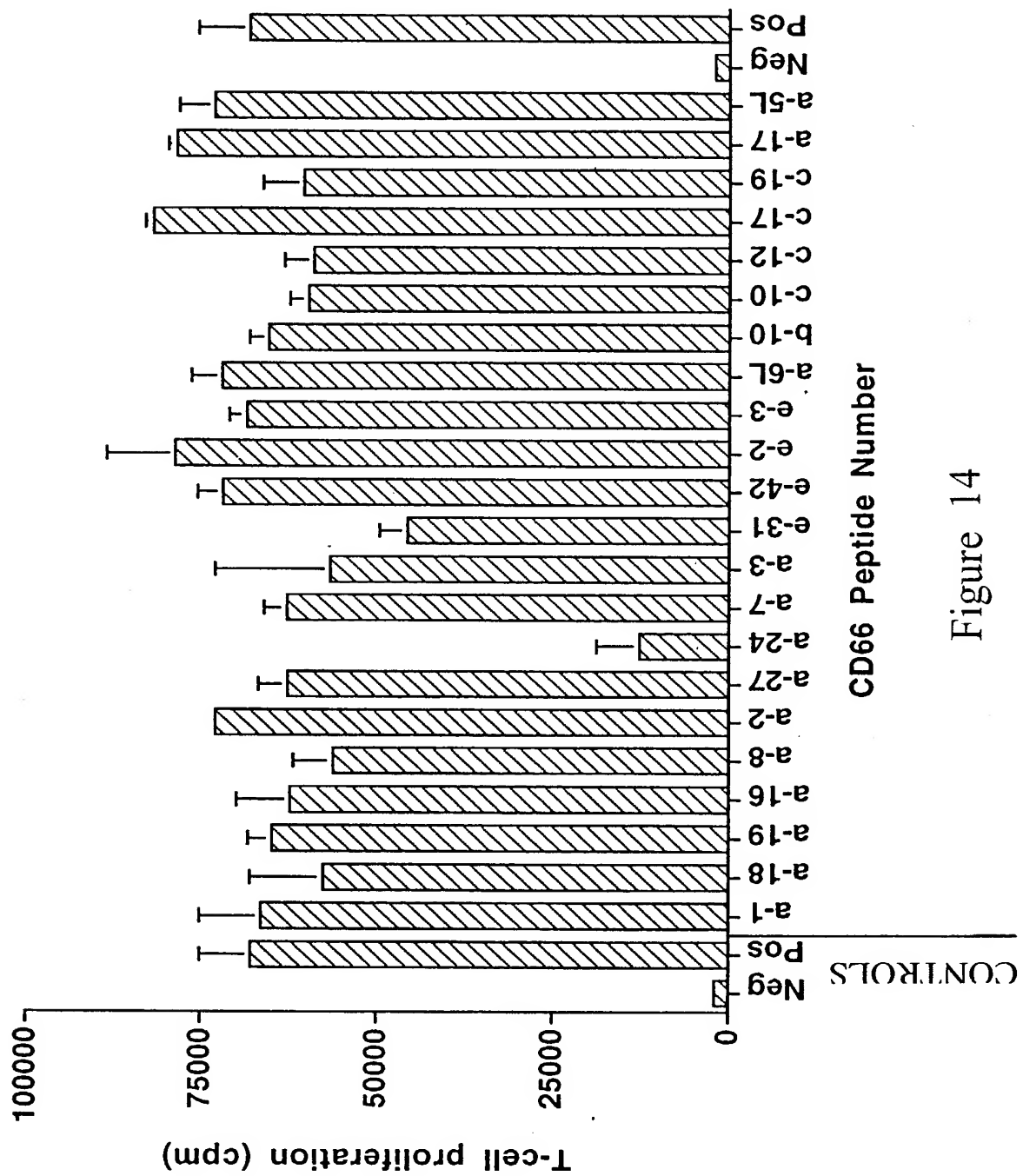


Figure 13B

19/19



Docket No: 284.00010101

DECLARATION AND POWER OF ATTORNEY

We, Keith M. Skubitz and Amy P.N. Skubitz, declare that: (1) our respective citizenships and residence/mailling addresses are indicated below; (2) we have reviewed and understand the contents of the specification identified below, including the claims, as amended by any amendment specifically referred to herein, (3) we believe that we are the original, first, and joint inventors of the subject matter in

PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

Filing Date: Herewith

Serial No.: Unassigned

described and claimed therein and for which a patent is sought; and (4) we hereby acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56.*

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below, or §365(a) of any PCT international application which designates at least one country other than the United States of America listed below, and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on the basis of which priority is claimed:

a. ☒ no such applications have been filed.b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC §119(a)-(d), §365(a), and/or §365(b)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

*Title 37, Code of Federal Regulations, §1.56 is reproduced on the attached page.

Declaration and Power of Attorney

Page 2 of 4

Serial No. Unassigned

Filing Date: Herewith

Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

We hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

- a. no such applications have been filed.
b. X such applications have been filed as follows:

PROVISIONAL APPLICATION(S), IF ANY, UNDER 35 USC §119(e)	
APPLICATION NUMBER	DATE OF FILING (day, month, year)
60/150,791	26 August 1999 (26.08.99)
60/152,501	2 September 1999 (02.09.99)

We hereby claim the benefit under Title 35, United States Code, §120 of any United States applications or §365(c) of any PCT international application(s) designating the United States of America, listed below.

- a. no such applications have been filed.
b. X such applications have been filed as follows:

APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)
PCT/US00/23482	26 August 2000 (26.08.00)	pending

Insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

We hereby appoint Ann M. Mueeting (Reg. No. 33,977), Kevin W. Raasch (Reg. No. 35,651), Mark J. Gebhardt (Reg. No. 35,518), Victoria A. Sandberg (Reg. No. 41,287), David L. Provence (Reg. No. 43,022), Matthew W. Adams (Reg. No. 43,459), Loren Albin (Reg. No. 37,763), Kathleen L. Franklin (Reg. No. 47,574), and Joseph C. Huebsch (Reg. No. 42,673) our attorneys with full powers (including the powers of appointment, substitution, and revocation) to prosecute this application and any division, continuation, continuation-in-part, reexamination or reissue thereof, and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence in this case to:

Attention: Ann M. Mueeting
Mueeting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Telephone No. (612) 305-1217
Customer Number 26813

Declaration and Power of Attorney

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*Serial No. Unassigned**Filing Date: Herewith*Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Wherefore, we pray that Letters Patent be granted to us for the invention described and claimed in the specification identified above and we hereby subscribe our names to the foregoing specification and claims, Declaration, and Power of Attorney on the date indicated below.

Name Keith M. Skubitz

Citizenship: USA

Residence: 6704 Cahill Road, Edina, Minnesota 55439-1309

Mailing Address:

(If different than Residence)

Date

200 Name Amy P.N. Skubitz

Citizenship: USA

Residence: 6704 Cahill Road, Edina, Minnesota 55439-1309 MN

Mailing Address:

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Date Feb 26, 2002

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Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

§ 1.56 Duty to disclose information material to patentability.

- (1) Prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) The closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

- (1) Each inventor named in the application;
- (2) Each attorney or agent who prepares or prosecutes the application; and
- (3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(e) In any continuation-in-part application, the duty under this section includes the duty to disclose to the

Office all information known to the person to be material to patentability, as defined in paragraph (b) of this section, which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

Docket No: 284.00010101

DECLARATION AND POWER OF ATTORNEY

We, Keith M. Skubitz and Amy P.N. Skubitz, declare that: (1) our respective citizenships and residence/mailling addresses are indicated below; (2) we have reviewed and understand the contents of the specification identified below, including the claims, as amended by any amendment specifically referred to herein. (3) we believe that we are the original, first, and joint inventors of the subject matter in

PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

Filing Date: Herewith

Serial No.: Unassigned

described and claimed therein and for which a patent is sought; and (4) we hereby acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56.*

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below, or §365(a) of any PCT international application which designates at least one country other than the United States of America listed below, and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on the basis of which priority is claimed:

a. ☒ no such applications have been filed.b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC §119(a)-(d), §365(a), and/or §365(b)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

* Title 37, Code of Federal Regulations, §1.56 is reproduced on the attached page.

Declaration and Power of Attorney

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Serial No. Unassigned

Filing Date: Herewith

Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

We hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

- a. ☐ no such applications have been filed.
b. ☒ such applications have been filed as follows:

PROVISIONAL APPLICATION(S), IF ANY, UNDER 35 USC §119(e)	
APPLICATION NUMBER	DATE OF FILING (day, month, year)
60/150,791	26 August 1999 (26.08.99)
60/152,401	2 September 1999 (02.09.99)

We hereby claim the benefit under Title 35, United States Code, §120 of any United States applications or §365(c) of any PCT international application(s) designating the United States of America, listed below.

- a. ☐ no such applications have been filed.
b. ☒ such applications have been filed as follows:

APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)
PCT/US00/23482	26 August 2000 (26.08.00)	pending

Insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

9 We hereby appoint Ann M. Mueiting (Reg. No. 33,977), Kevin W. Raasch (Reg. No. 35,651), Mark J. Gebhardt (Reg. No. 35,518), Victoria A. Sandberg (Reg. No. 41,287), David L. Provence (Reg. No. 43,022), Matthew W. Adams (Reg. No. 43,459), Loren Albin (Reg. No. 37,763), Kathleen L. Franklin (Reg. No. 47,574), and Joseph C. Huebsch (Reg. No. 42,673) our attorneys with full powers (including the powers of appointment, substitution, and revocation) to prosecute this application and any division, continuation, continuation-in-part, reexamination or reissue thereof, and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence in this case to:

Attention: Ann M. Mueiting
Mueiting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Telephone No. (612) 305-1217
Customer Number 26813

Declaration and Power of Attorney

Serial No. Unsigned

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Filing Date: Herewith

Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS

The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Wherefore, we pray that Letters Patent be granted to us for the invention described and claimed in the specification identified above and we hereby subscribe our names to the foregoing specification and claims, Declaration, and Power of Attorney on the date indicated below.

100 Keith M. Skubitz
Name Keith M. Skubitz

Citizenship: USA

Residence: 6704 Cahill Road, Edina, Minnesota 55439-1309 *MN*

Mailing Address:

(If different than Residence)

2-26-02
Date

Name Amy P.N. Skubitz

Citizenship: USA

Residence: 6704 Cahill Road, Edina, Minnesota 55439-1309

Mailing Address:

(If different than Residence)

Date

Declaration and Power of Attorney

Serial No. Unassigned

Filing Date: Herewith

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Title: PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66 (CEACAM) FAMILY MEMBERS**§ 1.56 Duty to disclose information material to patentability.**

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) Prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) The closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

- (1) It establishes, by itself or in combination with other information, a *prima facie* case of unpatentability of a claim; or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

A *prima facie* case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

- (1) Each inventor named in the application;
- (2) Each attorney or agent who prepares or prosecutes the application; and
- (3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

(e) In any continuation-in-part application, the duty under this section includes the duty to disclose to the Office all information known to the person to be material to patentability, as defined in paragraph (b) of this section, which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : Skubitz et al.
SERIAL NUMBER : 10/069,605 EXAMINER : Not yet assigned
FILING DATE : February 26, 2002 ART UNIT : Not yet assigned
FOR : PEPTIDES CAPABLE OF MODULATING THE FUNCTION OF CD66
(CEACAM) FAMILY MEMBERS

Assistant Commissioner for Patents
Washington, D.C. 20231

REVOCATION BY APPLICANTS AND NEW POWER OF ATTORNEY

Keith M. Skubitz and Amy P.N. Skubitz, Applicants of record for the above-identified United States patent application Serial No. 10/069,605, filed February 26, 2002, hereby revoke any and all former powers of attorney and appoint:

Attorney or Agent	Registration No.	Attorney or Agent	Registration No.
Kevin Ainsworth	39,586	Shane Hunter	41,858
Ingrid Beattie	42,306	David E. Johnson	41,874
William Belanger	40,509	Christina Karnakis	45,899
Charles E. Bell	48,128	Kristin E. Konzak	44,848
Naomi Biswas	38,384	Cynthia Kozakiewicz	42,764
Bradford C. Blaise	47,429	Barry J. Marenberg	40,715
Sean M. Coughlin	48,593	Scott D. Miller	43,803
David F. Crosby	36,400	A. Jason Mirabito	28,161
Christopher J. Cuneo	42,450	Michel Morency	50,183
Brett N. Dorny	35,860	Carol H. Peters	45,010
Marianne Downing	42,870	Matthew Pavao	50,572
Ivor R. Elrifi	39,529	Michael Renaud	44,299
Heidi A. Erlacher	45,409	Robert J. Sayre	42,124
Eugene Feher	33,171	C. Eric Schulman	43,350
John M. Garvey	37,833	Gregory J. Sieczkiewicz	48,223
James G. Gatto	32,694	Thomas M. Sullivan	39,392
Richard Gervase	46,725	Janine Susan	46,119
Matthew J. Golden	35,161	Nicholas P. Triano III	36,397
Sonia K. Guterman	44,729	Eric Sinn	40,177
Paul Hayes	28,307	Howard Sussner	33,556
Brian P. Hopkins	42,669		

APPLICATION No. 10/069,605
APPLICANTS: Keith M. Skubitz *et al.*

all of MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO PC, One Financial Center, Boston, Massachusetts 02111, as Applicants' attorneys with full power of substitution and revocation to take any and all action necessary with regard to the above-identified patent application.

SEND CORRESPONDENCE TO:
Matthew J. Golden, Esq.
Mintz Levin
One Financial Center
Boston, MA 02111

DIRECT TELEPHONE CALLS TO:
Matthew J. Golden, Esq.
(212) 692-6818

We, the undersigned, hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the patent.

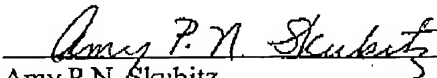
Please charge any fee or any fee deficiency occasioned by this document to Deposit Account No. 50-0311, Attorney Reference No. 25210-011 NATL.

Respectfully submitted,

Date: 7-26-02


Keith M. Skubitz

Date: July 24, 2002


Amy P.N. Skubitz